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Role of elevated phosphorus on wheat plant under different split-root systems

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Role of elevated phosphorus on wheat plant under different split-root systems

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Statement of Authorship

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CERTIFICATE

Certified that this thesis, entitled ' **Role of elevated phosphorus on wheat plant under different split-root systems'** is a record of research work done independently by **Mrs. Rubya Shabnam** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associate ship to her.

Professor Dr. Md. Toufiq Iqbal
Supervisor

DEDICATED

TO

My Family

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The author

Abstract

Phosphorus (P) is a key nutrient for plant growth and development. It is involved in cellular energy transfer, respiration and photosynthesis. Nutritional requirements of plants vary widely mostly under different ecological conditions. Most of the plants have to survive under adverse to the most severe conditions as they are genetically adapted to their habitats and even some varieties of the same species differ very much in absorption translocations, accumulation and nutrient use. The P is taken up from the soil solution by plant roots as orthophosphates ions, principally as mono-orthophosphates, $H_2PO_4^-$ ions in acidic soil solution while in alkaline soil solutions the principal ions that adsorbed/absorbed are monovalent orthophosphate, $H_2PO_4^-$ ions and divalent orthophosphate, HPO_4^{2-} ions.

In my several experiments, I have used two hypes of soils- one acidic soil having initial soil pH 5.2 in water and the other alkaline soil having initial soil pH 7.9 in water. Two recently BARI released wheat varieties namely BARI GOM 25 and BARI GOM 26 were used throughout the investigations. The KH_2PO_4 was used as a source of P. The experiments were carried out to examine different parameters such as the roles and effects of different P levels and application methods with three triplications in improving root and shoot developments of wheat plants (in split-root systems), plant growth and development by wheat seedlings, phosphorus use efficiency (PUE) by wheat plants that grown in both acidic and alkaline soils. Effects of added soluble P on the rate of absorption/adsorption of nutrients from the soil solutions and also the effect of P supply to the crop by the soil available P, P fertilizer management, soil and environmental conditions that influence P apply to availability and root-shoot ratio.

The experimental results along with their comparison between BARI GOM 25 and BARI GOM 26 in acidic and alkaline soil which in turn show the betterment of wheat plant growth in acidic soil over alkaline soil in tabular and graphical forms.

In acidic soil, results showed that the growth parameter plant biomass and P uptake increased 90%-91% with respect to the controlled treatment. Slightly better results but not highly significant, were found for BARI GOM 26 than those for BARI GOM 25. The findings clearly indicate that elevated P takes a significant part in wheat plant development and the added soluble P increases the absorption of nutrients from both acidic and alkaline solutions and the application of elevated P is efficient for both increasing shoot development and the root growths and the PUE with respect to plant utilization. Results also showed that alkaline soil are somewhat below than therefor acid soil. Moreover, the 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 that means, the translocation of P in the roots in acidic and alkaline soils and there is no doubt that P plays a significant role in the P dynamics and P translocation within the wheat plants in split root systems (in both acidic and alkaline soils).

The overall experimental results suggested that the acid soil is more suitable than alkaline soil for BARI GOM 25 and BARI GOM 26 wheat (*Triticum aestivum* L) cultivation. The study concluded that the both wheat varieties can able to utilize P efficiently in both acidic and alkaline soils with the overcoming of P fixation in both soils.

Abbreviations and Acronyms

AAS	Atomic Absorption Spectrophotometer
Al	Aluminium
ANOVA	Analysis of Variance
AR	Analytical Reagent
BARI	Bangladesh Agriculture Research Institute
C	Compartment
CRD	Completely Randomized Design
CSIRO	Commonwealth Scientific and Industrial Research Organization
DAS	Days after sowing
DAT	Day after Transplanting
DI	De-ionized
DTPA	Diethylenetriamine Pentaacetic Acid
FAO	Food and Agricultural Organization
G	Grams
hr	Hour
K	Potassium
L.	Linneaus (Carolus Linneaus)

N	Nitrogen
P	Phosphorus
PUE	Phosphorus Use Efficiency
T	Treatment
UK	United Kingdom
V	Variety

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Chapter 1

General introduction

Chapter 1

1. General Introduction

1.1 Background

Wheat (*Triticum aestivum* L.) is not only the most important cereal crops but also a major source of staple food for the Bangladeshi people. It is now become the secondary food supplement also for cattle, fishes and poultry throughout the world. Despite being grown on nearly 40% of the total arable land of the Bangladesh, the average yield at the farmers' field is much below the potential. Traditional method of seed-bed preparation, late planting after rice, high weeds and pathogens infestations, poor irrigation and water shortage at the critical stages of growth and development and non-judicious use of fertilizers particularly phosphorus (P) limit the wheat productivity. As P is a micronutrient, and wheat plants commonly suffer very much from P deficiency while grown in alkaline and acidic soils. It is, therefore, imperative to manage P properly to achieve maximum benefits over 90% of Bangladeshi soils are low in soil available P and suffer from moderate to severe P deficiency, where wheat plants require adequate P from the very early stages of growth for optimum crop production. According to some estimates, 5.7 billion hectares of the world's arable lands have an insufficient amount of available P to maintain optimum crop production (Batjes, 1997). Unlike other macronutrients, the P concentration in the soil solution is often very low and ranges from 2 to 10μM (Raghothama, 1999; Brady and Weil, 2002). In addition, due to the unique properties of the interaction of P with other elements, up to 80% of added P fertilizer can be fixed in the soil (Holford, 1997).

Among the many factors that affect the P availability soil pH is the most important one, as solubility of the phosphate compounds are directly related to the soil pH. The P availability is limited both in acidic and alkaline soils. Most studies confirm that P mobility is the greatest between pH 6.0 to 6.5. It is well-known that at lower and higher pH, P can form the insoluble phosphate compounds. The P fertilization is a very important factor for high yield achievement (Lott *et al.*, 2011). Soils prone to P fixation need larger amounts of P fertilizer. Ameliorative fertilization or applications of higher P amounts can improve the soil fertility of P deficient soils and increase crops yields (Petošić *et al.*, 2003). Some researches indicate that residual effect of P lasts longer than that of potassium (Kadar *et al.*, 2010). Wheat have high phosphorus requirement, especially during the early growth stages, as P affects rooting and tillering. The P deficiency could limit the wheat yield by reducing the number of ears per area due to a poor tiller emergence (Rodriguez *et al.*, 1999).

Plant P efficiency is broadly defined as having relatively greater biomass at less optimal P level (Lynch, 1998; Liao *et al.*, 2008), including P acquisition efficiency and P utilization efficiency, which could be separately reflected by P content and biomass produced by unit P in plants (Graham, 1984; Clark and Duncan, 1991; Batten, 1992). Since P is rarely mobile in soils, P acquisition efficiency is mainly determined by the soil volume explored to the roots as indicated by root morphology (i.e. root length and root surface area) and root architecture i.e. the spatial distribution of roots along soil profile (Yan and Zhang, 1997). Accumulated results reveal that changes of root traits lead to increase of P acquisition efficiency, including modifying root morphology and architecture, activating high affinity of P fertilizer (Raghothama,

1999). All these studies imply that root traits are vital for plants to acquire P efficiently from the soils under various combinations of P applied conditions.

1.2 Scope of work

Phosphorus (P) is involved in several key plant functions including energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant and transfer of genetic characteristics from generation after generation (Pasek 2008). The P availability is one of the major growth limiting factors in many ecosystems around the world (Barber *et al.*, 1963). Its availability is limited both in acidic and alkaline soils. Phosphate is found in a low concentration in soil solution, as such plant high-affinity inorganic P transporters are responsible for all the P that moves into the plant, and subsequently all of the P found in living organisms (Oelkers and Valsami-Jones, 2008). As P deficiency is also a critical nutritional problem in plant growth so it 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 the plants, as orthophosphate ($H_2PO_4^-$ and HPO_4^{2-}) forms (Hinsinger, 2001). Phosphorus is important in several physiological processes of plants, especially in photosynthesis, carbon metabolism and membrane formation (Wu *et al.*, 2005). 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 formation of insoluble complexes with cations. This means that inorganic P is often a limiting factor in plant growth and development. 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). Phosphorus is readily translocated within the plants, moving from older to younger tissues as the plant forms cells and develops roots, stems and leaves. 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. To understand the mechanisms controlling these traits is, therefore, of great importance in the pursuit of improved crop inorganic P uptake.

1.3 Aims of the research

Most of the studies on P application on wheat plant have been conducted in nutrient solutions and rooting media which are substantially different from P acquisition, translocation and utilization by wheat plant. The primary mechanisms involved in the utilization of phosphorus by wheat plant, which means, P uptake, distribution and transport in plants, are not thoroughly understood. Existing solution experiments may not be appropriate to understand mechanisms of P utilization by wheat plants. Therefore, in this thesis different experimental studies have been conducted to understand the P utilization mechanisms by wheat plants with following aims:

I. To understand how elevated P behaves in both acidic soil and alkaline soil.

II. To evaluate growth response of recently BARI released wheat plant under elevated P applied condition in different split-root systems.

III. To examine the phosphorus status and phosphorus distribution in the different parts of wheat plant in different split-root systems under both acidic and alkaline soil condition.

IV. To understand mechanisms involved in the utilization of inorganic P by wheat plant under various split-root systems.

V. To quantify how translocated P effects on wheat plant within split-root system under P efficient condition.

1.4 Organization of thesis

This thesis consists of ten chapters. Apart from the current "General Introduction" chapter, the remaining of the thesis has been divided into nine chapters.

Chapter 2 “Literature Review” provides an insight on the P acquisition, fixation, translocation and utilization by wheat plants in different soil plant conditions.

Chapter 3 "General Materials and Methods" gives an overview of our study methodologies.

Chapter 4 "Phosphorus use efficiency by wheat plants that grow in an acidic soil" is an experimental chapter that describes the effects of elevated P application on wheat plant that grown in an acidic condition.

Chapter 5 "Effect of elevated phosphorus on growth response to wheat plant that grow in an alkaline soil" is an experimental chapter that describes the effects of elevated P application on wheat plant that grown in an alkaline condition.

Chapter 6 "Understanding phosphorus dynamics in wheat plant and growth response in a split-root system in acidic soil" is an experimental chapter that describes the P dynamics in wheat plant in a split-root system in acidic condition.

Chapter 7 "Understanding phosphorus dynamics in wheat plant and growth response in a split-root system in alkaline soil" is an experimental chapter that describes the P dynamics in wheat plant in a split-root system in alkaline condition.

Chapter 8 "Understanding phosphorus translocation in wheat plant and growth response in a split-root system" is an experimental chapter that describes the P translocation and growth responses of wheat plant in both acidic and alkaline soil under several split-root systems.

Chapter 9 “General Discussion” that presents the overall discussion on elevated P application on wheat plant under different split-root systems.

Chapter 10 “Conclusion and Recommendation” that presents the summery of the study on elevated P application on wheat plant under different split-root systems and future work from this study.

Chapter 11 “References” that represent the whole reference of the thesis.

Chapter 2

Literature Review

Chapter 2

2. Literature Review

2.1 Introduction

Wheat, an annual plant of Poaceae family, belongs to the genus *Triticum* which has 18 species of which bread wheat (*Triticum aestivum*) is the most important in Agriculture. Among the cereal crops, wheat ranks first in world consumption but second in Bangladesh after rice.

Wheat provides larger contribution of proteins and minerals to humans and animals than all other cereal crops. Walton stated that wheat is the most important source of concentrated carbohydrate for human being and it contains considerable amounts of proteins, minerals and vitamins.

Wheat is considered as the king of cereal crops because its cultivation is easier, nutrient content is higher and ecologically suitable. For normal growth and development, wheat crop requires macro and micronutrients in addition to its proper management of cultivations.

Phosphorus (P) is an essential macro nutrient. It is vital to plant growth and is found in every living plant cell. It is involved in several key plant functions including energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant and transfer of genetic characteristics from one generation to the next. Low P availability is one of the major factors limiting crop production in both acidic and alkaline soils.

2.2 Soil–plant interactions

Phosphorus (P) is taken up from the soil solution by plant roots as orthophosphate ions, principally as monovalent orthophosphate, $H_2PO_4^-$ ions and to a lesser extent as divalent orthophosphate, HPO_4^{2-} ions. Several factors can influence on both the rate and amount of P taken up by the plant and, therefore, can affect the recovery of a single application of P fertilizer. The same factors can also affect the recovery of P reserves accumulated in the soil from the past additions of P as fertilizer or manure.

The most important factors controlling the availability of P to plant roots are its concentration in the soil solution and the P-buffer capacity of the soil. The latter controls the rate at which P in the soil solution is replenished, i.e. the rate of desorption of P from the solid phase of the soil, which is faster in soils with a high buffer capacity. Also important are the size of the root system and the extent to which roots grow into the soil, and the efficiency with which roots take up P. When considered a single application of P fertilizer, its efficiency depends on how well it is mixed with the volume of exploited by the roots. Other factors that affect the crop yield, include P uptake by the crop, recovery of P and its influence and efficiency include soil moisture and the extent of control of weeds, pests and diseases i.e., crop management. Because the effects of these factors vary from year to year, it is essential to average estimates of P recovery over a number of years in order to obtain reliable data.

2.3 Concentration of phosphorus in the soil solution

The concentration of P in the soil solution ranges from 10^{-4} M to 10^{-6} M, deficient, to as low as 10^{-8} M in some very low-fertility tropical soils. These concentrations can be

related to the amount of P in the soil solution and crop uptake of P. For example, a concentration of 10^{-5} M corresponds to 0.31 mg P per litre in the soil solution. Assuming that the top 30 cm of soil holds 6 cm of water (equivalent to 600 m^3 per hectare) there will be less than 0.2 kg P ha^{-1} in the soil solution to that depth. If a crop uses 37 cm of water during its growth, there will only be about 1 kg P ha^{-1} dissolved in the soil solution, yet it may take up 20–40 kg P ha^{-1} during the growing season. This much larger uptake is possible because roots can absorb P from solutions with very small P concentrations and P is maintained in solution by desorption from the solid phase of the soil. Provided that there is sufficient P on adsorption sites, from which it can be desorbed readily, and that the rate of release is adequate, plants will obtain enough P to meet their changing demand during the growing season. The rate of P release is an important factor (Frossard *et al.*, 2000), but it is difficult to measure routinely because radioisotopes and expensive counting equipment are required.

The minimum concentration of P to which the roots of soil-grown plants can deplete the external concentration of P in the rhizosphere soil solution is about 1 µM (Hendriks, Claassen and Jungk, 1981). The amount of P in the bulk soil solution required to replenish this concentration of P in the root hair cylinder can be estimated as follows. If the concentration of P is 5 µM, equivalent to 0.15 mg P per litre, and the amount of solution in the top 30 cm of soil is 500 000 litres per hectare, then the quantity of P is 0.075 kg ha^{-1}. However, the crop requirement for P during its phase of rapid growth can range between 0.3 and 0.5 kg P ha^{-1} per day. To meet this requirement for P during the period of maximum demand, the P in the root hair cylinder has to be replenished at least 10–20 times each day. This is because roots explore only about 25 percent of the topsoil in any one growing season (Jungk, 1984), but this depends on the crop grown.

2.4 Soil acidity problem for crop production

Soil acidity is a major constraint to crop production. Soil acidification is a continuous process and is accelerated by factors such as acid rain and the excess application of ammonium-based fertilisers (Zheng 2010). The primary limitations for crop production on acid soils are toxic levels of aluminium (Al) and manganese (Mn), as well as suboptimal levels of P (Kochain *et al.* 2004). Of these limitations, Al toxicity has been recognized as a major constraint for crop production in acid soil (Yang *et al.* 2005). The toxic Al^{3+} ions in acid soils restrict crop production by reducing either the availability of essential nutrients or the effectiveness of the roots to obtain nutrients and moisture (Tang *et al.* 2001). Similarly, the accumulation of toxic Al^{3+} within the plant tissue affects the plant growth and development in acid soil (Ritchey *et al.* 1991). Another limitation is the shortage of P that occurs due to the decrease in soluble P in the soil in presence of high Al so that P deficiency is often associated with Al toxicity in acidic soil. As a result, both Al toxicity and P deficiency limit crop production in acid soils (Sanchez *et al.* 1997). Therefore, it is important to understand how P interacts with Al on plant growth in acid soils so that a feasible and cost-effective method to alleviate the acidity problem may be proposed.

2.5 Soil alkalinity problem for crop production

Alkali, or alkaline, soils are clay soils with high pH (> 8.5), poor soil structure and low infiltration capacity. Often they have a hard calcareous layer at 0.5 to 1 m depth. Alkali soils owe their unfavorable physico-chemical properties mainly to the dominating presence of sodium carbonate which causes the soils to swell [Managing

irrigation water quality, Oregon State University, USA, Retrieved on 2012-10-04.] and difficulty to clarify/settle. This is important because high alkalinity exerts the most significant effects on growing medium fertility and plant nutrition.

Alkaline soils are difficult to take into agricultural production. Due to the low infiltration capacity, rain water stagnates on the soil easily and, in dry periods, cultivation is hardly possible without copings irrigated water and good drainage. Agriculture is limited to crops tolerant to surface waterlogging (e.g. rice, grasses) and the productivity is low.

Phosphorus (P) availability is one of the major growth limiting factors in many ecosystems around the world (Barber *et al.*, 1963). Large amounts of P fertilizers are generally required for sustainable crop production on variable charge soils because of low P availability to plants (Barrow, 1986; Lin, 1995). In sandy soils of arid area of southern east Algeria, P is influenced by various factors such as alkaline (pH>7) soil conditions and the high $CaCO_3$ content (>3%). Regular applications of ordinary superphosphate to sandy soils in laboratory leaching experiments led to a buildup of acid extractable inorganic P even though more than 80% of P in the fertilizer is lost during the leaching phase following application (Ritchie and Weaver, 1993).

Due to these interactions, nearly 80% of applied P as fertilizers may be fixed in the soil (Barrow, 1980; Holford, 1997). According to Raghothama *et al.* (2005) and Rahim *et al.* (2007), P deficiency is very common in alkaline calcareous soils. The

amount of soil P removed by crops need to be replenished through the application of fertilizer P and manure to maintain soil P balance (Saleque *et al.*, 2006).

2.6 Effect of pH on P availability

Low P availability in acid soils is a major limiting factor for plant growth (Nian *et al.* 2009). Generally, P availability in soil is minimum in the pH range of 2 to 4 (Bowden *et al.* 1980). In low-pH soils, the formation of Al-phosphate decreases the concentration of P in the soil solution that is controlled by precipitation reactions (Pratt 1961). In addition, Schefe *et al.* (2007) reported that low soil pH reduces the availability of P also through increased P sorption reaction.

The fixation of P depends on soil pH (Hsu 1964). The fixation onto colloidal iron (Fe) surfaces beginning at very low pH levels, usually below 4, is the dominant P fixation process in soils (Kanwar and Grewal 1990; Naidu *et al.* 1990). There is a relationship between soil pH and P fixation (Hocking *et al.* 1999). Soil pH below 5.5 affects solubility of P in soils characterized by cracking clays, where Al and Fe dominate. The P fixation with Al is more commonly seen from pH 4.5 to 6 and results in substantial lock-up of P, while in less acid-to-neutral pH soils, calcium (Ca) phosphate is the more commonly encountered inorganic form of P. Above the pH level 7.0, Ca is the dominant ions and fixation is less permanent. The fixation of P in acid, neutral and alkaline soil due to Fe, Al and Ca is shown in (Figure 2-1).

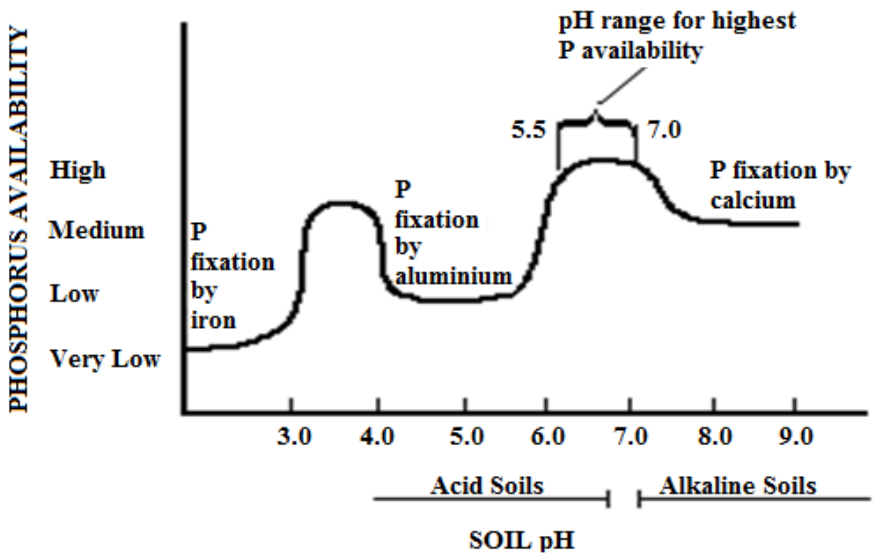

Figure 2-1: The fixation and availability of P is affected by soil pH (Source: CSIRO, 2006)

2.7 Movement of phosphorus to the roots

Plant root systems have two main functions; first, to provide an anchor for the plant in the soil, and second, to take up water and nutrients from the soil solution. Roots do not grow throughout the whole volume even of the surface soil and, as noted above, roots explore perhaps as little as 25 percent of the topsoil in one growing season. Roots can intercept nutrients (Barber *et al.*, 1963) but less than 1 percent of the available soil nutrients are supplied in this way (Barber, 1984). Nutrients are taken up from the soil in the region of the root, and this process is largely dependent on nutrients moving to the root by two distinct processes, mass flow and diffusion (Barber, 1984).

The amount of nutrient transported by mass flow is related to the amount and rate of water movement to the root, the water use by the crop, and the concentration of the nutrient in the soil solution. For example, assuming the concentration of P in the soil

solution is 0.15 mg per litre and a crop transpires 3 million litres of water per hectare during its growth, then the total amount of P delivered to the roots is about 0.45 kg P per hectare. This quantity is only 2–3 percent of the total amount of P required by many crops to produce acceptable yields.

Diffusion is the main process by which P moves to the root surface. Diffusion involves the movement of ions along a concentration gradient, i.e. from a higher to a lower concentration. Thus, when plant roots remove nutrient ions from the soil solution and the concentration is lowered relative to that in the bulk solution, a concentration gradient develops and nutrient ions move down this gradient. The extent of depletion at the root surface depends on the balance between the supply from the soil and the demand by the plant. If the "absorbing power" of the root is large, this creates a sink to which nutrients diffuse (Tinker and Nye, 2000). The root-absorbing power is not constant but depends on root metabolism and the nutrient status of the plant (Barber, 1984). The amount of P required at the root surface depends on the depletion profile that develops with time. The shape of this profile will depend on the balance between P uptake by roots, the rate at which P is replenished in the soil solution, and the mobility of the phosphate ions by diffusion.

The mobility of an ion is defined in terms of a diffusion coefficient, which is usually orders of magnitude smaller in soils than in homogeneous media, such as water, because of the tortuosity (complexity of shape and length) and small diameter of most water-filled pores in the matrix of the heterogeneous soil system. Marschner (1995) gives estimated diffusion coefficients of $H_2PO_4^-$ (the most common form of inorganic orthophosphate in solution in weakly-acid aqueous systems) in water as 0.9×10^{-9} m^2s^{-1}, but in soil estimated values range from 10^{-12} to 10^{-15} m^2s^{-1}. This very limited

movement of phosphate ions explains why it is necessary to have a sufficient supply of readily-available P throughout the volume of soil explored by roots if the demand for P by a crop is to be met during its most active period of growth. It also explains why good responses are often obtained by placing P fertilizer near where the roots of a crop are expected to grow.

2.8 Plant root systems and phosphorus uptake by roots

Plant roots take up P from the soil solution as orthophosphate ions, principally $H_2PO_4^-$ and to a lesser extent HPO_4^{2-}, except in calcareous and saline soils. Plant roots can absorb P from soil solutions having very low P concentrations (Loneragan and Asher, 1967), in which case P uptake is against a very steep P concentration gradient. This is because the P content of root cells and xylem sap is 100–1000 times larger than that of the soil solution (Mengel and Kirkby, 1987). The transport of P across the cell membrane varies between plant species. Cultivars within the same species can differ in their capacity for active P uptake, and these differences are probably largely genetically controlled.

Many plants have extensive root systems, which frequently have root hairs that extend out into the rhizosphere (the cylinder of soil surrounding the root), thereby increasing the effective surface area of the root system for the uptake of water and nutrients. Root hair formation is modified by environmental factors such as nutrient supply, especially that of N and P, and it differs between species. In non-mycorrhizal plants, the extent of the zone of P depletion in the soil as a result of active P uptake by roots is often closely related to root hair length. For example, the extent of the P-depletion zone around maize and oilseed rape roots is nearly the same as the maximum root hair

length, 1.8 mm for maize and 2.6 mm for rape, respectively (Hendriks, Claassen and Jungk, 1981). Itoh and Barber (1983) found a strong positive correlation between P-uptake rate per unit root length and the volume of the root hair cylinder. Caradus (1982) also showed differences in the efficiency of P uptake between genotypes of white clover that were related to root hair length.

Root hairs are more effective in absorbing P than is the root cylinder when the influx per unit area of each is compared because the smaller diameter and geometric arrangement of the root hairs maintain higher diffusion rates for P (Jungk and Claassen, 1989; Claassen, 1990). In soils with little readily-available P, uptake by root hairs can account for up to 90 percent of total P uptake by the plant (Föhse *et al.*, 1991).

However, a close relationship between root hair length and the extent of the P-depletion zone in the rhizosphere is not always found. For example, the P-depletion zone around cotton, with short root hairs (about 0.2 mm) greatly exceeds the root hair cylinder (Misra *et al.*, 1988). For nonmycorrhizal plants, this suggests root-induced changes in the rhizosphere, e.g. the release of root exudates (particularly low-molecular-weight organic acids), pH changes, or a higher efficiency of uptake per unit length of root.

Many plants have developed a symbiotic association with arbuscular mycorrhizal (AM) fungi. The spores, which are found in many soils, develop hyphae that penetrate the root, remove carbohydrates from it, and grow out into the soil immediately surrounding the root, extending the capacity of the root to take up water and nutrients, especially P and micronutrients (Tinker, 1984). In soils with adequate plant-available P, this fungal association is usually not well developed, suggesting that mycorrhizae

are not important in such soils. In mycorrhizal plants, the extent of the P-depletion zone greatly exceeds the diameter of the root hair cylinder (Jungk and Claassen, 1989), and it can be as large as 11 cm in white clover (Li *et al.*, 1991). Some plants do not have a mycorrhizal association. These include species of the order Chenopodiaceae, which includes agriculturally-important crops such as sugar beet. Compared with crops with mycorrhizae, such crops have the disadvantages considerably when grown on soils with very small amounts of readily-available P (Johnston *et al.*, 1986).

Differences between genotypes in P-use efficiency may be caused by differences in P uptake by roots, P transport within roots, P transport from root to shoot and between organs within shoots, and the utilization of P within the plant (Marschner, 1995). Perhaps the most important factor causing differences between genotypes is the acquisition of P by roots. Differences in P uptake per unit root length may be caused by higher influx rates, longer root hairs or differences in root/shoot ratios relative to the availability of P in the soil solution. As these differences are genetically controlled, there should be good prospects for developing more P-efficient genotypes. If such genotypes become available, it should be possible to maintain soils at lower critical P concentrations than those required for current cultivars. Such P-efficient genotypes, whether produced by conventional breeding techniques or genetic manipulation, would have to be high-yielding and not more susceptible than current cultivars to other nutrient deficiencies and abiotic and biotic stress. Brown, Clark and Jones (1977) showed that some P-efficient genotypes are more susceptible to iron (Fe) and copper (Cu) deficiencies.

There may be reasonably good prospects for improving the efficiency of P use by plants by selecting appropriate genotypes with characteristics for root hair length,

organic acid production in the rhizosphere, and mycorrhizal associations for soils with low P status. This approach to improving P-use efficiency may be more appropriate than seeking to modify root architecture, i.e. the shape and branching of the root system, which is often suggested as a way of improving nutrient uptake. Field evidence shows that root distribution in soil is much more dependent on soil physical characteristics than on the inherent shape of the root system. Almost 37 years ago, Drew and Saker (1978) showed that plant roots proliferated in soil zones that are enriched in P rather than following some specific pattern of spatial distribution.

2.9 P translocation in whole plant

Several researcher like Mimura *et al.*, 1996; 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 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). A

schematic representation of the possible mechanisms of P acquisition and translocation is given in Figure 2-2.

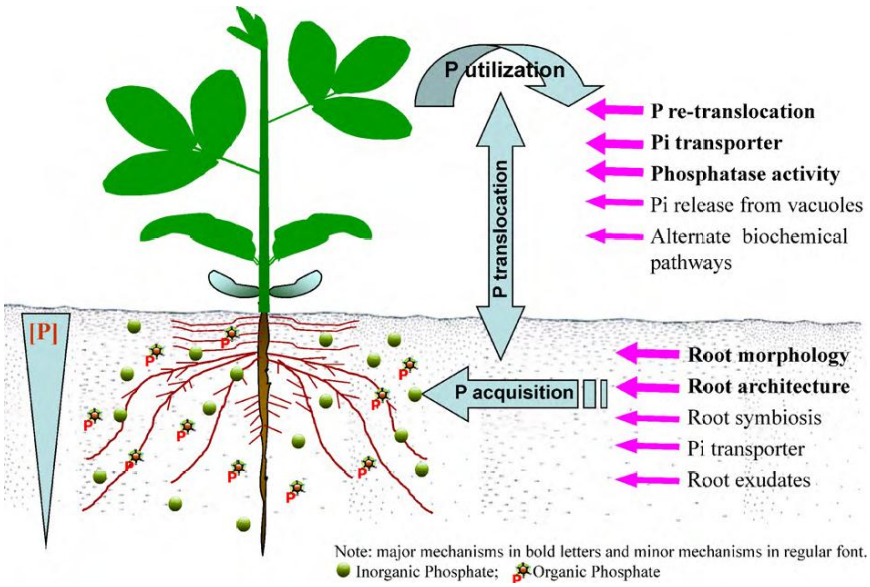

Figure 2-2: Schematic representation of the possible mechanisms of P acquisition, translocation and utilization

The phosphate status of the shoot is known to control the root processes involved in inorganic P uptake and its subsequent translocation; however, the precise mechanisms of this control are unknown). The retranslocation of phosphate from the shoot to the root is likely to be involved, acting as a feedback-regulatory mechanism responsible for mediating the shoot control of phosphate levels within the plant (Drew and Saker, 1984; Schjørring and Jense´n, 1984; Marschner and Cakmak, 1986). Feedback mechanisms regulating the movement of ions between the root and the shoot have also been proposed for sulfate (Jense´n and Ko¨ nig, 1982) and potassium (Pitman, 1977). Retranslocated inorganic P and organic derivatives of phosphate (Hall and Baker, 1972) may have dual functions both as nutrients for root processes and as signaling molecules (Drew and Saker, 1984). The precise points of regulation in the root are unclear, although some evidence implies that the loading of inorganic P in the

xylem is central to the regulation of phosphate flux in the plant (Drew and Saker, 1984). The Arabidopsis mutant pho1 (Poirier *et al.*, 1991) exhibits normal inorganic P uptake rates from the soil to the root, but is blocked in the loading of inorganic P into the xylem, which is genetic evidence for at least partial regulation of inorganic P transport by xylem loading.

Plants respond to decreasing inorganic P in the environment by increased root growth, increased expression of inorganic P transporters (Muchhal *et al.*, 1996; Leggewie *et al.*, 1997; Liu *et al.*, 1998b), and alterations in metabolism, including secretion of acid phosphatases (Lefebvre *et al.*, 1990) and RNAses (Bariola *et al.*, 1994), which assist in the liberation of inorganic P from the rhizosphere. Internally, phosphate retranslocation from the shoot to the root increases (Lefebvre *et al.*, 1990; Heuwinkel *et al.*, 1992; Jeschke *et al.*, 1997). Recently, however, it has been shown that in inorganic P-starved plants, a larger percentage of retranslocated phosphate is returns to the shoot, indicating that phosphate cycling occurs (Jeschke *et al.*, 1997).

2.10 Concluding remarks

Phosphorus is important in several physiological processes of plants, especially in photosynthesis, carbon metabolism, and membrane formation (Wu *et al.*, 2005). 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. This means that inorganic P is often a limiting factor in plant growth and development. Most of the studies on P application on wheat plant have been conducted in nutrient solutions and rooting media which are substantially different from P acquisition, translocation and utilization by wheat plant. The primary mechanisms involved in the utilization of phosphorus by wheat plant, which means, P

uptake, distribution and transport in plants are not thoroughly understood. However, little is known about the mechanisms involved in utilization and translocation of P on wheat plant; we have used soil-grown plants and isogonic wheat varieties to study the phosphorus (P) status in the different part of wheat (*Triticum aestivum* L.) plant and the effects of elevated P on wheat plant growth and P acquisition, translocation and utilization in a split-root soil culture.

Chapter 3

General Materials and Methods

Chapter 3

3. General Materials and Methods

Experimental materials and methodologies which are commonly used throughout this research are outlined below. Modifications or specific techniques are further described in the Materials and Methods section of each relevant chapters.

3.1 Soils

Two types of soils (acidic and alkaline) were used in this study. The soil type I (acidic) having pH 5.2 was collected from acidic region of Bangladesh and the soil type II (alkaline) having pH 7.9 was collected from alkaline soil region of Bangladesh. The basic properties of soil are out-lined in Table 3.1.

Table 3.1: Properties of soils used in different experiments

Soil Type	Soil pH	Total N (%)	Available P (ppm)	Exchangeable K (Cmol/Kg)	Available S (ppm)	Available Zn (ppm)	Organic matter (%)
Soil I (Acidic)	5.2	0.05	10.2	0.2	19.5	0.59	0.85
Soil II (Alkaline)	7.9	0.03	14.3	0.21	5.6	11.55	0.55

3.2 Plants

The BARI GOM 25 and BARI GOM 26 wheat (*Triticum aestivum* L.) varieties were used as testing plants. Among them, BARI GOM 25 is suitable for optimum and late

sowing conditions and moderately tolerant to salinity up to 2 dS/m, highly tolerant to Bipolaris leaf blight and resistant to leaf rust diseases. The BARI GOM 26 is suitable for optimum and late sowing conditions - tolerant to heat stress.

3.3 Seed germination

Seeds of uniform size were selected for germination. The seeds of the BARI GOM 25 and BARI GOM 26 were germinated in moist sand in two separate petri dishes in dark at 25°C for 70h. Five holes (1.0cm deep and 1.0 cm wide) were made in the soil in each plastic cup containing 200g pre-incubated soil. Keeping the radicals in down ward direction five pre-germinated seeds of BARI GOM 25 and BARI GOM 26 were placed carefully in those holes in each cup and then the seeds were gently covered with the same treated soil. BARI GOM 25 and BARI GOM 26 were placed in separate cups. After sowing, each cup was covered with filter paper for first two (2) days to avoid disturbance of top soil. Deionized (DI) water was sprayed on the filter paper to keep the soil moist. To maintain the required soil moisture content 20 ml DI water was added every day to each cup during the growth period of wheat plants and watering was stopped 3 days before harvesting.

Figure 3-1: A view of plant growth experiment, showing BARI GOM 25 and BARI GOM 26

3.4 Plant growing condition

Plants were grown in an open air under 10h dark and 14h light conditions. The variations of day and night temperature were 20-30°C and 15-20°C respectively. All the cups were re-randomized in their position on alternate days during the growing period of wheat seedlings, to minimize the positional effects.

3.5 Plant harvesting

Plants were harvested at 28 days after sowing. Whole plants with roots and surrounding soil were removed from each cup by gentle agitating to provide minimum disturbance to the roots and shoots. Intact plants were then lifted up gently from the

soil and shaken lightly to remove the bulk soil and then washed to remove the adhered soil from roots. Collected bulk soil was air-dried and stored in a refrigerator until analysis. Shoots and roots were separated. Shoots were oven dried at 70°C for 3 days and stored for analysis. The harvested roots were washed with DI water and oven-dried at 70°C temp for 3 days and stored for analysis.

3.6 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 was continued for 28 days.

Figure 3-2: A view of split-root system, showing two compartments with BARI GOM 25 and BARI GOM 26 in Treatment C at 20 DAT

3.6.1 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 into consideration. 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. A 20 ml of DI water was added to each compartment every day and watering was stopped at 3 days before harvesting.

3.6.2 Harvesting

The experimental plants were harvested 28 days after transplanting. The shoots were cut into 0.5 cm above the base part of the stem uniformly. Then the roots were cut 0.5 cm below the base part and separated carefully into two halves as previously marked. Soils from two root halves removed carefully so that the roots could not be toned 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 over dried at the same temp for the same time. After drying, the root and shoot samples were weighed and stored for analytical experiments.

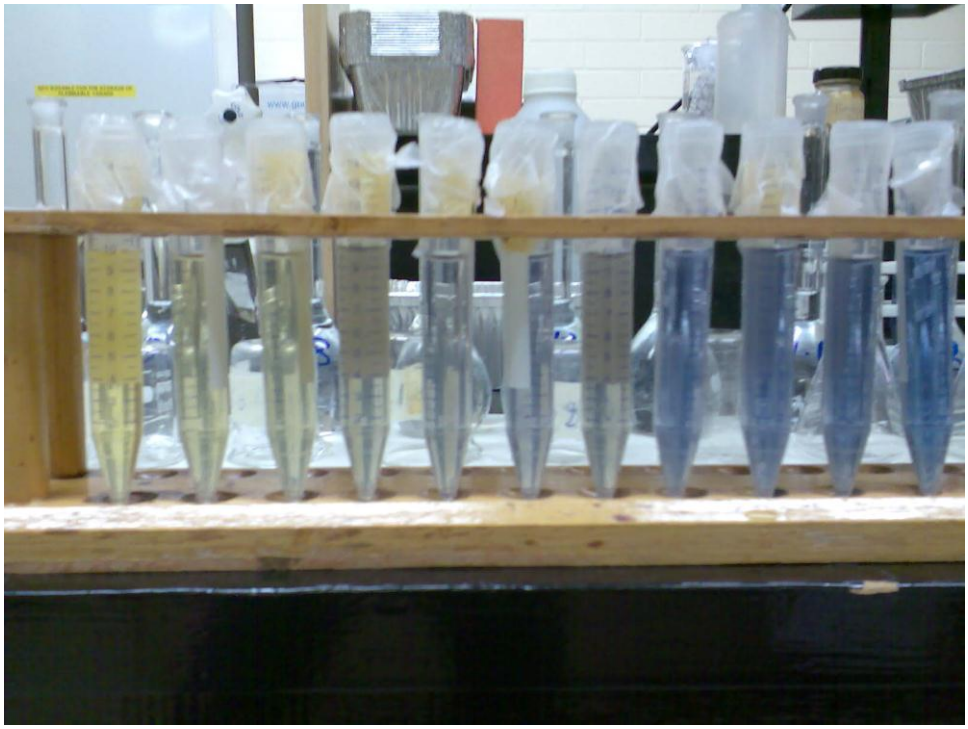

Figure 3-3: Root and shoot samples prepared for analytical experiments

3.7 Stock solution preparation for P source

Mono-potassium di hydrogen phosphate (KH_2PO_4) was used as P source. According to the treatment of each of the experiments, the total amount of mono-potassium di hydrogen phosphate was calculated for different rates and diluted with DI water in a volumetric flask. The diluted mono-potassium di hydrogen phosphate solution was pipetted instantly into the soil to avoid precipitation of P within the diluted solution.

3.8 pH measurements

Soil pH was determined with a 1:5 extraction in 0.01 M $CaCl_2$. A 5 g sample of air-dried soil was mixed with 25-ml 0.01 M $CaCl_2$ in 50-ml plastic vials and was shaken mechanically overnight (16 hr). Vials were centrifuged for 30 minutes at 3500 rpm to

recover soil solution. Soil pH was measured, after allowing tubes to stand 5 minutes, using a glass electrode and a Thermo Orion 720 pH meter.

3.9 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 percloric acids (4:1), the concentration of P in root and shoot materials were determined using the vanadomolybdate method. Colorimetric method for the determination of P 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 DI water. The absorbance reading was used at 470 nm (Iqbal *et al.*, 2010).

3.9.1 Phosphorus use efficiency calculation

Phosphorus use efficiency, PUE was calculated using the formulae as described by Fageria *et al.*, (1997).

$$PUE\% = \frac{Total\ P\ uptake\ \left(\frac{mg}{kg}\right) in\ Treatment\ pot - Total\ P\ uptake\ \left(\frac{mg}{kg}\right) in\ control\ pot}{P\ dose\ applied\ (\frac{mg}{kg})} \times 100$$

Total P uptake (mg/kg) = P uptake by root (mg/kg) + P uptake by shoot (mg/kg)

3.9.2 Measurements of soil physical and chemical properties

Soil textural analysis was conducted by using an abbreviated version of the international pipette method. Clay content was determined by 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 experiment 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 Asija, 1956). The distillate was collected in 20 ml of 2% boric acid solution with methylred and bromocresol green indicator and titrated with 0.02 N sulphuric acid (H_2SO_4) (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 NH_4OAC and determined by an atomic absorption spectrometer (Biswas *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 $NaHCO_3$ 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).

3.9.3 Statistical analysis

Shoot and root parameters were analysed by two-way ANOVA (Treatment × Varieties), 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 (Lawes Agricultural Trust, UK).

3.10 Quality control

The analytical method validation was confirmed with fortification and recovery study. All chemicals and standards were AR or GR grade and soil as well as plant samples analysis were verified with reference samples throughout the study period. Two methods (molydovanado-phosphate and malachite green) were used for determination of P in plant tissue to verify the results. If unexpected results were found during measurement, two to three time measurements were accomplished to confirm the results.

Chapter 4

Phosphorus use efficiency by wheat plants that grown in an acidic soil

Chapter 4

4. Phosphorus use efficiency by wheat plants that grown in an acidic soil

4.1 Introduction

Phosphorus (P) is an essential macro nutrient. It is vital to plant growth and is found in every living plant cell. It is involved in several key plant functions including energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant and transfer of genetic characteristics from generation after generation (Pasek 2008). Low P availability is one of the major factors limiting crop production in acidic soils.

Phosphorus is taken up from the soil solution by plant roots as orthophosphate ions, principally as monovalent orthophosphate, $H_2PO_4^-$ ions and to a lesser extent divalent orthophosphate, HPO_4^{2-}ions (Armstrong 1999). Several factors can influence on both the rate and amount of P taken up by the plant and therefore, can affect the recovery of a single application of P fertilizer. The same factors can also affect the recovery of P reserves accumulated in the soil from the past additions of P as fertilizer or manure (Syers *et al.*, 2008).

Syers *et al.*, (2008) also reported in FAO fertilizer and plant nutrient bulletin that the most important factors controlling the availability of P to plant roots are its

concentration in the soil solution and the P-buffer capacity of the soil. The latter controls the rate at which P in the soil solution is replenished, i.e. the rate of desorption of P from the solid phase of the soil, which is faster in soils with a high buffer capacity. Also important are the size of the root system and the extent to which roots grow into the soil, and the efficiency with which roots take up P. When considered a single application of P fertilizer, its efficiency depends on how well it was mixed with the volume of exploited by the roots. Other factors that affect the crop yield, P uptake by the crop, recovery of P and its influence and efficiency include soil moisture and the extent of control of weeds, pests and diseases i.e., crop management. Because the effects of these factors vary from year to year, it is essential to average estimates of P recovery over a number of years in order to obtain reliable data.

This study aims with the following objectives: to understand how elevated P behaves in an acidic soil, to determine P uptake by recently BARI released wheat plant under acidic condition, evaluate growth response of recently BARI released wheat varieties under elevated P applied condition. This study hypothesized that elevated P will help to utilized more P by the wheat plant even in acidic condition.

4.2 Materials and Methods

4.2.1 Soil and Plant

Acidic soil collected form Thakurgaon district, Bangladesh was used as experimental soil. The initial soil pH was 5.2. The basic properties of soil are out-lined in Table 4.1.The BARI GOM-25 and BARI GOM-26 wheat varieties were used as a testing plant.

Table 4.1: Soil basic physical and chemical properties used in this experiment

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

4.2.2 Experimental design

The CRD (completely Randomized Design) was adopted. The CRD experiment consisted of five levels of Phosphorus (P)–0, 30, 60, 90 and 120 mg/kg P and two wheat varieties- BARI GOM 25 and BARI GOM 26 with three replications. The KH_2PO_4 was used as P source. To avoid the interactions between soil nutrients and added P no basal nutrients were added. The plants allowed growing 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 30^0C for 7 days then KH_2PO_4 as per P doses were 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 & Agricultural Extension, Rajshahi University, Rajshahi, Bangladesh.

4.2.3 Seed germination and plant sowing

Seeds of uniform size were selected for germination. The seeds BARI GOM-25 and BARI GOM-26 were germinated in moist sand in two separate petri dishes in dark at 25^0C for 70h. Five holes (1.0 cm deep and 1.0 cm wide) were made in the soil in each

plastic cup containing 200g pre-incubated soil. Keeping the radicals in down ward direction five pre-germinated seeds of BARI GOM 25 and BARI GOM 26 were placed carefully in those holes in each cup and then the seeds were gently covered with the same treated soil. The BARI GOM 25 and BARI GOM 26 were placed in separated cups. After sowing, each cup was covered by filter paper for first two (2) days to avoid disturbance of top soil. Deionized (DI) water was sprayed on the filter paper to keep the soil moist. To maintain the required soil moisture content 20 ml DI water was added every day to each cup during the growth period of wheat plants and watering was stopped at 3 days before harvesting.

4.2.4 Plant growth condition

Plants were grown in open air under 10h dark and 14h light conditions. The variations of day and night temperature were 20-30°C and 15-20°C respectively. All the cups were re-randomized in their position on alternate days during the growing period of wheat seedlings, to minimize the positional effects.

4.2.5 Plant harvesting

Plants were harvested 27 days after sowing (DAS). Whole plants with roots and surrounding soil were removed from each cup by gentle agitating to provide minimum disturbance to the roots and shoots. Intact plants were then lifted up gently from the soil and shaken lightly to remove the bulk soil and then washed to remove the adhered soil from roots. Collected bulk soil was air-dried and stored in a controlled room

temperature (25°C) until analysis. Shoots and roots were separated. Shoots were oven dried at 70°C for 3 days and stored for analysis. The harvested roots were washed with DI water and oven-dried at 70°C temp for 3 days and stored for analysis.

4.3 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 percloric acids (4: 1), the concentration of P in root and shoot materials were determined. Concentration of P in root and shoot materials were determined using the vanadomolybdate method after digestion in a mixture of concentrated nitric and perchloric acids (4:1). Colorimetric method for the determination of P 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 DI water. The absorbance reading was used at 470 nm (Iqbal *et al.*, 2010).

4.3.1 Statistical analysis

Shoot and root parameters were analysed by two-way ANOVA (Treatment × Variety), 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 (Lawes Agricultural Trust, UK).

4.3.2 Phosphorus use efficiency calculation

Phosphorus use efficiency was calculated using the formula as described by Fageria *et al.*, (1997).

$$PUE\% = \frac{Total\ P\ uptake\ \left(\frac{mg}{kg}\right) in\ Treatment\ pot - Total\ P\ uptake\ \left(\frac{mg}{kg}\right) in\ control\ pot}{P\ dose\ applied\ (\frac{mg}{kg})} \times 100$$

Total P uptake (mg/kg) = P uptake by root (mg/kg) + P uptake by shoot (mg/kg)

4.3.3 Measurements of soil physical and chemical properties

Soil textural analysis was conducted by using an abbreviated version of the international pipette method. Clay content was determined by 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 experiment 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 Asija, 1956). The distillate was collected in 20 ml of 2% boric acid solution with methylred and bromocresol green indicator and titrated with 0.02 N sulphuric acid (H_2SO_4) (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 NH_4OAC and determined by an atomic absorption spectrometer (Biswas *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 $NaHCO_3$ 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).

4.4 Results

All the plant growth and P-uptake parameters were highly significant ($P \leq 0.001$) under P levels (Table 4.2). Similarly, significant differences among varieties were observed in relation to all the growth and P-uptake parameters.

Table 4.2. Significance levels for the main and interactive effect of P and wheat 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)	***	n.s.	**	n.s.	**
T×V	***	n.s.	-	n.s.	-

Where n.s., ** and *** represent probability of > 0.05, ≤ 0.01 and ≤ 0.001, respectively.'-' indicates no data available.

4.4.1 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 4-1. The results showed for the variety BARI GOM 25 that the maximum plant height (34.7 mm) was recorded in treatment T5 (120 mg/kg P), while it was minimum (25.49 mm) in treatment T1 (control). Again, the results showed for the variety BARI GOM 26 that the maximum plant height (34.93 mm) was recorded in treatment T5 (120 mg/kg P), while it was minimum (26.06 mm) in treatment T1 (control). Plant height was significantly ($P \leq 0.001$) affected among all the various P application and variety of wheat plant. It also increased with the increasing level of phosphorus application. Hence, among low level of various phosphorous applications, P had the gradual increasing effect on plant height with increasing P applications, while at high level P resulted in maximum plant height.

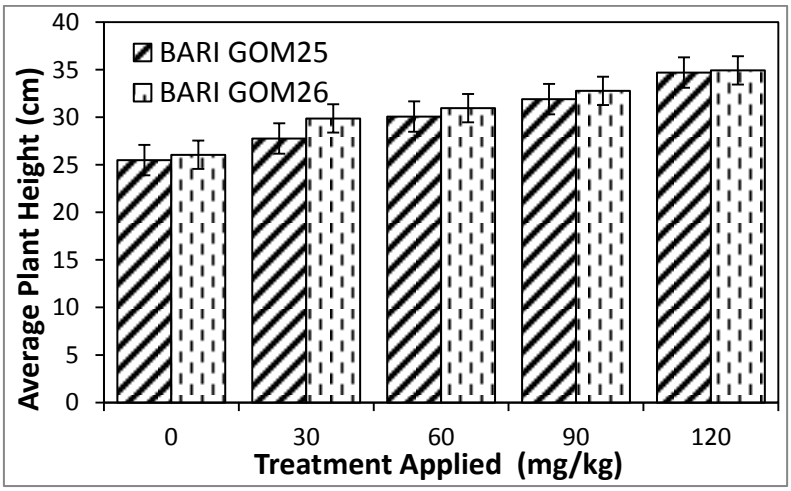

Figure 4-1: Effect of P application on average plant height of the wheat seedlings that was grown in various level of P for 28 days.

4.4.2 Shoot dry weight

As like plant height, the shoot biomass showed similar trend under different P applications. The results showed for the variety BARI GOM 25 that the maximum shoot biomass (0.85gm/plant) was recorded in treatment T5 (120 gm/kg P), while it was minimum (0.45gm/plant) in treatment T1 (control). Again, the results showed for the variety BARI GOM 26 that the maximum shoot biomass (0.87gm/plant) was recorded in treatment T5 (120 gm/kg P), while it was minimum (0.47 gm/plant) in treatment T1 (control). The shoot biomass was significantly ($P \leq 0.001$) affected among all the various P applications on wheat plant. Also, the shoot biomass did not significantly ($P>0.05$) differed between varieties of wheat plant. The shoot biomass also increased with the increasing level of phosphorus application (Figure 4-2).

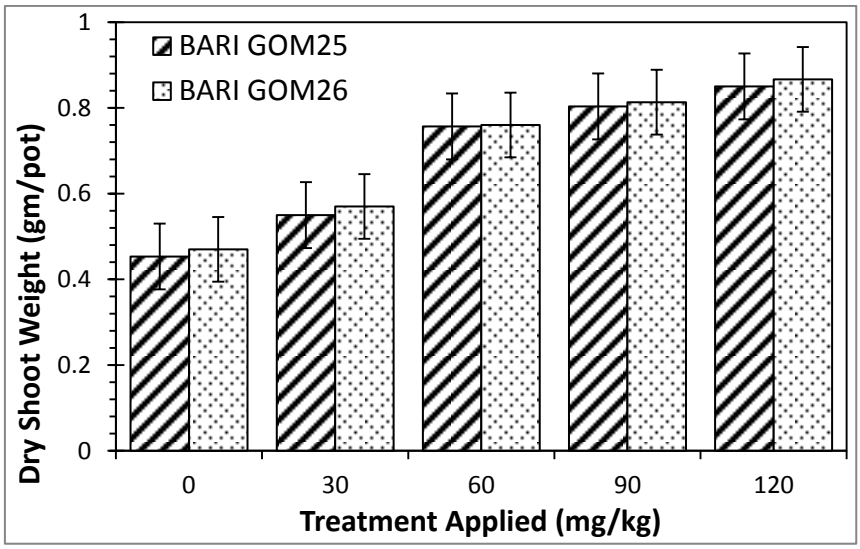

Figure 4-2: Effect of P application on dry shoot weight of the wheat seedlings that was grown in various level of P for 28 days.

4.4.3 Root dry weight

Total root biomass varied among all the treatments. Total root biomass in BARI GOM 26 were the highest and the lowest in Treatment T5 (0.67 gm/plant) and Treatment T1 (0.32 gm/plant) respectively, followed by gradual increase in Treatments T2 (0.42 gm/plant), T3 (0.56 gm/plant) and Treatment T4 (0.61 gm/plant) (Figure 4-3). Again, for BARI GOM 25 total root biomass were the highest and the lowest in Treatment T5 (0.65 gm/plant) and Treatment T1 (0.30 gm/plant) respectively, followed by gradual increase in Treatments T2 (0.40 gm/plant), T3 (0.55 gm/plant) and Treatment T4 (0.60 gm/plant). Similar to shoot dry weight, root biomass was significantly ($P \leq 0.001$) affected among all the various P application on wheat plant. But, the root biomass did not significantly ($P>0.05$) differed between varieties of wheat plant. The root biomass also increased with the increasing level of P application (Figure 4-3).

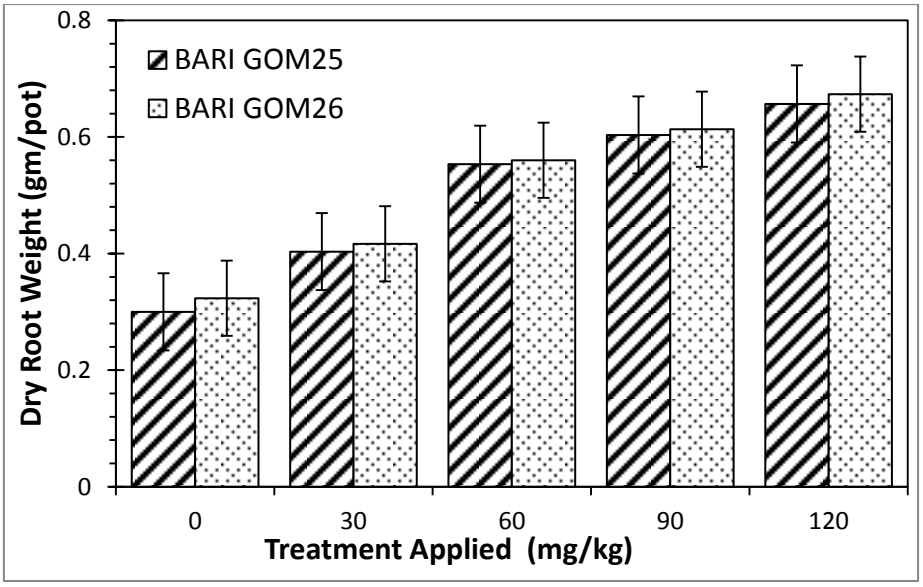

Figure 4-3: Effect of P application on dry root weight of the wheat seedlings that was grown in various level of P for 28 days.

4.4.4 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 were the highest and the lowest in Treatment T5 (4.53 gm/kg) and Treatment T1 (0.41 gm/kg) respectively, followed by gradual increase in Treatments T2 (1.36 gm/kg), T3 (3.87 gm/kg) and Treatment T4 (4.44 gm/kg) (Figure 4-4). Again, for BARI GOM 25 total shoot P concentration were the highest and the lowest in Treatment T5 (4.49 gm/kg) and Treatment T1 (0.40 gm/kg) respectively, followed by gradual increase Treatments T2 (1.29 gm/kg), T3 (3.79 gm/kg) and Treatment T4 (4.37 gm/kg).The shoot P concentration of BARI GOM 25 and BARI GOM 26 was dependent on the treatments. However, the two varieties had similar responses on shoot P concentration in the different treatments (Figure 4-4).

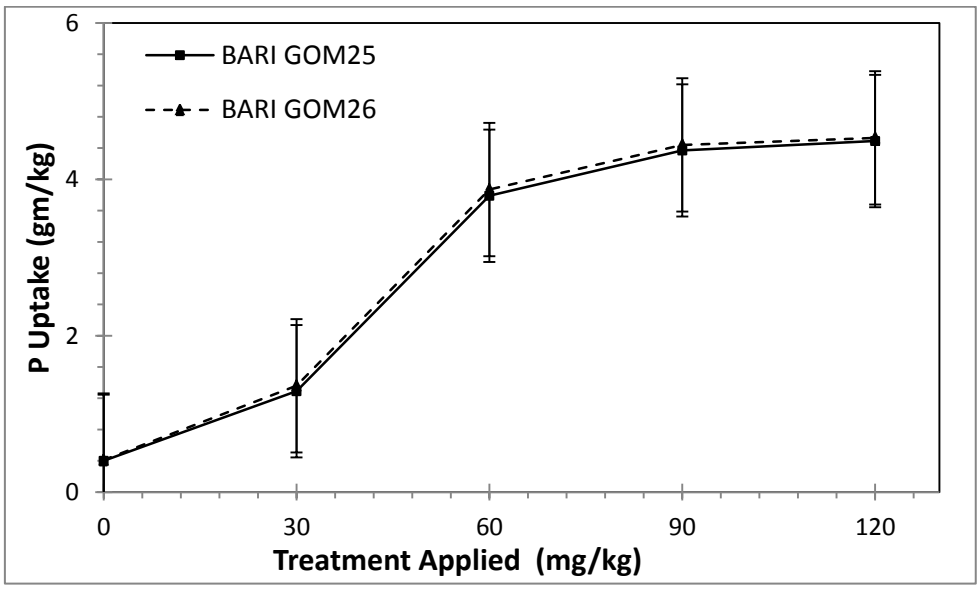

Figure 4-4: Effect of P application on P uptake of wheat shoot in various level of P for 28 days.

4.4.5 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 application on wheat plant. Total root P concentration in BARI GOM 26 were highest and the lowest in Treatment T5 (2.11 gm/kg) and Treatment T1 (0.31 gm/kg) respectively, followed by gradual increase in Treatments T2 (0.66 gm/kg), T3 (1.57 gm/kg) and Treatment T4 (1.87 gm/kg) (Figure 4-5). Again, for BARI GOM 25 total root P concentration were the highest and the lowest in Treatment T5 (2.07 gm/kg) and Treatment T1 (0.27 gm/kg), followed by gradual increase Treatments T2 (0.63 gm/kg), T3 (1.53 gm/kg) and Treatment T4 (1.85 gm/kg). The root P concentration of BARI GOM 25 and BARI GOM 26 was depend on the treatments. However, the two varieties had similar responses on root P concentration in the different treatments (Figure 4-5).

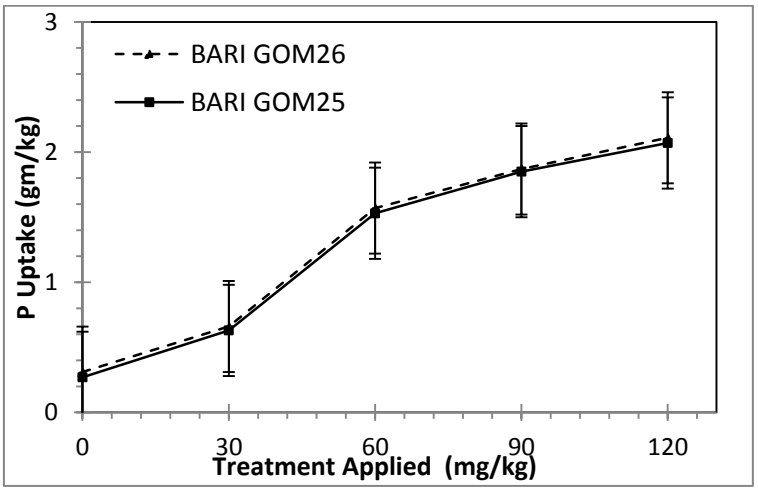

Figure 4-5: Effect of P application on P uptake of wheat root in various level of P for 28 days.

4.4.6 Total P uptake and P distribution

In both varieties of BARI GOM 25 and BARI GOM 26 were found similar trend in total P uptake. The total P uptake by wheat plant was significantly high in Treatment T5from other treatments in both varieties. However, total P uptake was about nine times greater in treatment T5 than control treatment T1. The total P uptake was greater in BARI GOM 26 than BARI GOM 25 in all treatments. Total P uptake in BARI GOM 26 were the highest in Treatment T5 (6.64 gm/kg) and the lowest in Treatment T1 (0.72 gm/kg) respectively, followed by gradual increase in Treatments T2 (2.02 gm/kg), T3 (5.44 gm/kg) and Treatment T4 (6.31 gm/kg) (Figure 4-6). Again, for BARI GOM 25 total P uptake were the highest in Treatment T5 (6.56 gm/kg) and the lowest in Treatment T1 (0.67 gm/kg) respectively, followed by gradual increase in Treatments T2 (1.92 gm/kg), T3 (5.32 gm/kg) and Treatment T4 (6.22 gm/kg) (Figure 4-6).

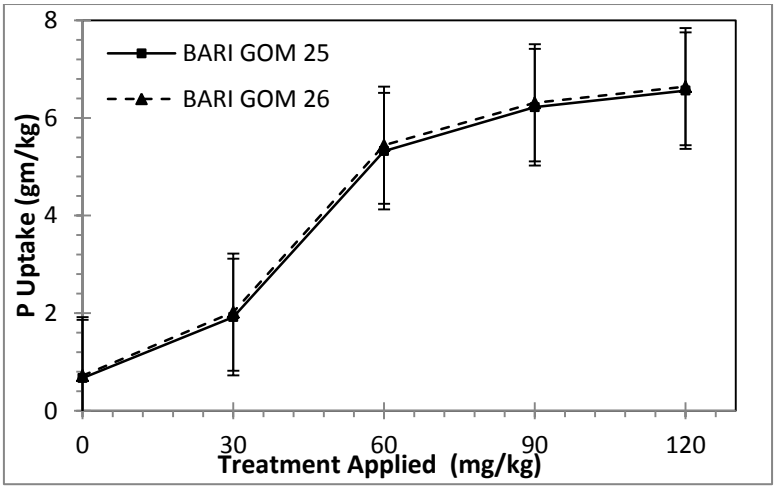

Figure 4-6: Effect of P application on P uptake of wheat plant in various level of P for 28 days.

4.4.7 P use efficiency in wheat plant

Data of P use efficiency (PUE) of wheat are given in Table 03. The PUE was calculated in terms of P uptake per unit of P application. The results revealed that lower PUE was seen at higher P rates. The maximum PUE of 7.75 % was observed at 60mg/kg P rate for variety BARI GOM 25 and it decreased significantly at higher P rates. Similarly, the maximum PUE of 7.95 % was observed at 60mg/kg P rate for variety BARI GOM 26 and it decreased significantly at higher P rates. Again, variety significantly affected the PUE and the minimum of 4.17 % was obtained with 30 mg/kg P application for variety BARI GOM 25 and 4.50 % was obtained with 30 mg/kg P application for variety BARI GOM 26. Results are in conformity with those of Rahim *et. al.*, (2010) who concluded that wheat growth increased significantly with the use of P. However, it is clear from Table 4.3 that P application through band placement significantly enhanced the PUE with that of broad casting.

Table 4.3: P concentration in root, P concentration in shoot and P use efficiency of two wheat varieties across different P levels

Variety	Treatment	P rate (mg/kg)	P in Shoot (gm/kg)	P in Root (gm/kg)	Total P uptake (gm/kg)	P use efficiency, PUE (%)
Bari GOM 25	T1	0	0.40	0.27	0.67	0.00
	T2	30	1.29	0.63	1.92	4.17
	T3	60	3.79	1.53	5.32	7.75
	T4	90	4.37	1.85	6.22	6.17
	T5	120	4.49	2.07	6.56	4.91
Bari GOM 26	T1	0	0.41	0.31	0.72	0.00
	T2	30	1.36	0.66	2.02	4.50
	T3	60	3.87	1.57	5.44	7.95
	T4	90	4.44	1.87	6.31	6.27
	T5	120	4.53	2.11	6.64	4.98

4.5 Discussion

4.5.1 Effects of elevated phosphorus application on acid soils

In general, plants grow better when partially soluble phosphate is applied in *comparison* to the soluble P source. Again, Rubio *et al.*, (2003) reported that the effect of pH change over P stability and this is on its availability soil pH influences. Soil pH influences the charge of the P species in solution as well as the charge of the adsorbing particles in soils. When pH rises, an increase in the negative charge is produced thereby decreasing P adsorption; but, at the same time, an increase in HPO_4^{2-} concentration is produced which has a much greater affinity for reactive soil surfaces which in turn increases P adsorption (Schachtman *et al.*, 1998). Whether a change in soil pH will increase or decrease P in soil solution sometimes depends on effect dominant (Whitelaw, 2000). Therefore, below pH 6.0, most P will be present as the monovalent $H_2PO_4^-$ species, whereas H_3PO_4 and HPO_4^{2-}will be present only in minor proportions. Most studies on the pH dependence of P uptake in higher plants have revealed that uptake rates are highest between pH 5.0 and 6.0, where $H_2PO_4^-$ dominates (Ullrich-Eberius *et al.*, 1984: Furihata *et al.*, 1992), which suggests that P is taken up as the monovalent form. The study was conducted in acidic soil and P doses were applied directly to the soil. The maximum Phosphorus use efficiency of 7.75 % was observed at 60 mg/kg P rate for variety BARI GOM 25 and it decreased significantly at higher P rates. Similarly, the maximum Phosphorus use efficiency of 7.95 % was observed at 60 mg/kg P rate for variety BARI GOM 26 and it decreased significantly at higher P rates. Results indicate that plant growth increased

significantly with the use of P. However, it is clear from Figure 4-7 that P application through band placement significantly enhanced the PUE with respect to broad casting.

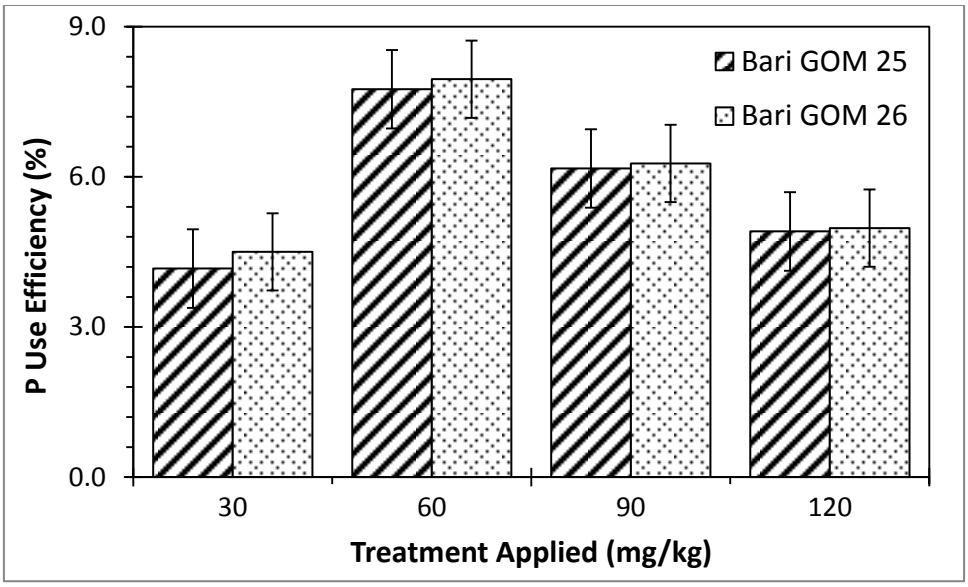

Figure 4-7: P use efficiency of two wheat varieties across different P levels

4.5.2 Phosphorus uptake efficiency by wheat plant under acidic soil condition

After harvesting, the shoot P uptake amount of the different treatments was compared. At low level of P supply, shoot P uptake was significantly increased in comparison with the controlled treatment (Figure 1). At high levels of available P, there was no significant difference among all treatments. Similar results were observed in different genotypes of wheat (Fageria and Baligar (1999); Ahmad *et al.*, (2013); Hu *et al.*, (2014)). Total shoot P concentration in BARI GOM 26 of Treatment T2 increased 70% (1.36 gm/kg) in comparison with the controlled Treatment T1 (0.41 gm/kg). Similar trend was found in T3 65% (3.87 gm/kg) in comparison of T2. But, at high levels of available P, in Treatment T4 and Treatment T5 the shoot P concentration increased 13% and 2 % respectively. Again, for BARI GOM 25 total soot P concentration in Treatment T2 was increased 69% (1.29 gm/kg) in comparison with

the controlled Treatment T1 (0.40 gm/kg). Similar trend was found in T3 66% (3.79 gm/kg) in comparison of T2. But, at high levels of available P, in Treatment T4 and Treatment T5 the shoot P concentration increased 13% and 3% respectively. This is in line with the suggestion of Cavagnaro *et al.*, (2003) that, P uptake by plant shoot was significantly high at low P concentration.

During collection, the root P uptake measures of the diverse treatments were analyzed. At low level of P supply, root P uptake was primarily found increase in examination with the controlled treatment (Figure 1). At elevated amounts of accessible P, there was no noteworthy contrast among all treatments. Comparative results were reported in different genotypes of wheat (Fageria and Baligar (1999); Ahmad *et al.*, (2013); Hu *et al.*, (2014)). Aggregate root P fixation in BARI GOM 26 of Treatment T2 increased 53% (0.66 gm/kg) in correlation with the controlled Treatment T1 (0.31 gm/kg). Comparable pattern was found in T3 58% (1.57 gm/kg) in correlation of Treatment T2. Yet, at high levels of accessible P, in Treatment T4 and Treatment T5 the root P fixation was expanded 16% and 11 % individually. For BARI GOM 25 aggregate root P concentration in Treatment T2 increased 57% (0.63 gm/kg) in examination compared with the controlled Treatment T1 (0.27 gm/kg). Comparative pattern was found in T3 59% (1.53 gm/kg) in examination of Treatment T2. However, at high levels of accessible P, in Treatment T4 and Treatment T5 the root P fixation increased 17% and 11 % separately. This is in accordance with the recommendation of Cavagnaro *et al.*, (2003) that, P uptake by plant root was significantly high at low P concentration.

The plant P uptake measures of the various treatments were investigated. Similar to the shoot and root phosphorus uptake pattern total plant phosphorus uptake results were compared with different treatments. Total plant P uptake in BARI GOM 26 of Treatment T2 increased 64% (2.02 gm/kg) in relationship with the controlled Treatment T1 (0.72 gm/kg). Similar example found in T3 63% (5.44 gm/kg) in connection of treatment T2. At high levels of accessible P, in treatment T4 and Treatment T5 the plant P uptake increased 14% and 5% independently. Again, for BARI GOM 25 total plant P uptake in Treatment T2 was extended 65% (1.92 gm/kg) in examination with the controlled Treatment T1 (0.67 gm/kg). Similar example found in T3 64% (5.32 gm/kg) in examination of Treatment T2. On the other hand, at large levels of available P, in Treatment T4 and Treatment T5 the plant P uptake was extended 14% and 5 % independently. Thus, P uptake by plant was on a very basic level high at low P fixation.

The relationship between shoot P uptake and average plant height was analyzed to investigate the P taken up by plants. A significant correlation (R^2= 0.87, $p < 0.05$) between plant height and shoot P uptake under elevated P supply indicated that plant development enhances with the application of P in soil (Figure 4-8). Similarly, a significant correlation (R^2 = 0.93, p<0.05) between plant height and root P uptake under elevated P application was observed. The relationship indicates that increase of the P uptake plant height increases with elevated P application which makes increasing absorption of nutrients from soil solution.

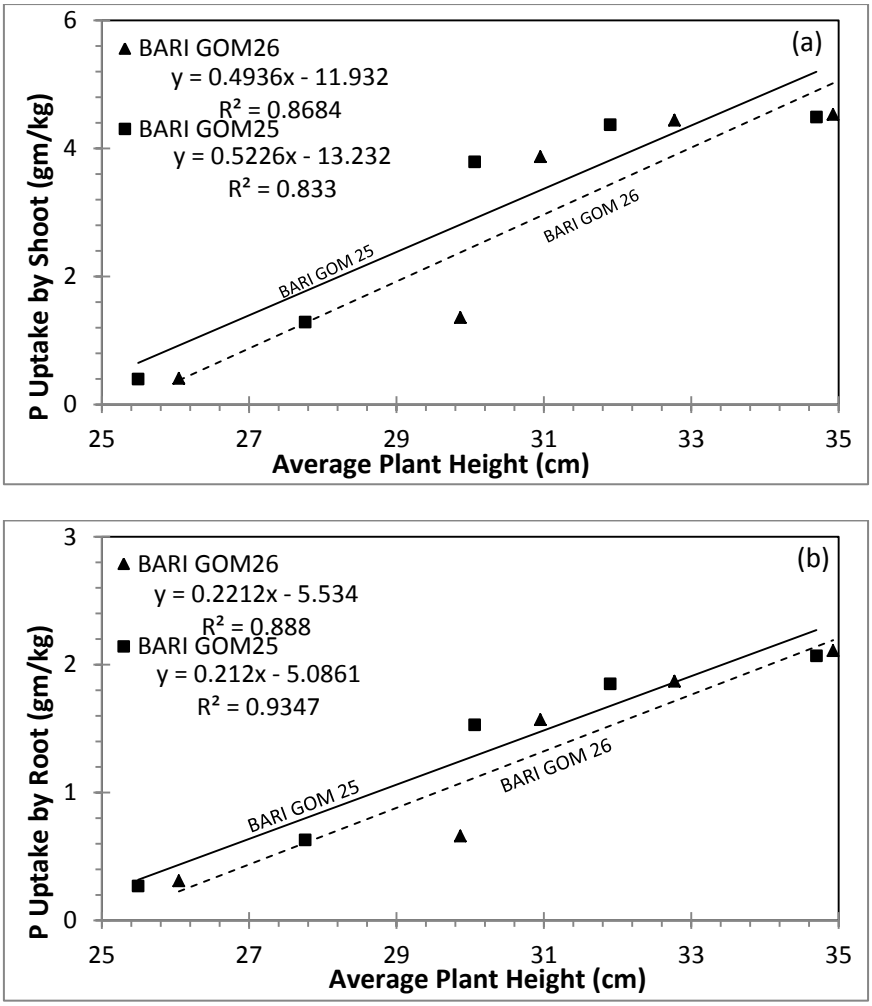

Figure 4-8: Relationship between (a) P uptake by shoot and average plant height; (b) P uptake by root and average plant height of wheat plant

4.5.3 Growth response of wheat plant for elevated P application in acid soils

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 shoot growth ($P \leq 0.001$) in this study indicates that the shoot growth responses of BARI GOM 25 and BARI GOM 26 seedlings were dependent on the level of added P. In all treatments, there were no significant differences between BARI GOM 25 and BARI GOM 26 seedlings for any growth measurement. The

results showed for the variety BARI GOM 25 that the maximum plant height (34.7 mm) was recorded in Treatment T5 (120 mg/kg P), while it was minimum (25.49 mm) in Treatment T1 (control). Again, the results showed for the variety BARI GOM 26 that the maximum plant height (34.93 mm) was recorded in Treatment T5 (120 mg/kg P), while it was minimum (26.06 mm) in Treatment T1 (control). Thus, plant height was significantly ($P \leq 0.001$) affected among all the various P application and variety of wheat plant.

The shoot biomass was also significantly ($P \leq 0.001$) affected among all the various P applications on wheat plant. The results showed for the variety BARI GOM 25 that the maximum shoot biomass (0.85 gm/plant) was recorded in Treatment T5 (120 gm/kg P), while it was minimum (0.45 gm/plant) in Treatment T1 (control). Again, the findings indicated for the variety BARI GOM 26 that the maximum shoot biomass (0.87 gm/plant) was in Treatment T5 (120 gm/kg P), while it was minimum (0.47 gm/plant) in Treatment T1 (control).

Investigation of another growth parameter showed that total root biomass varied among the treatments. Total root biomass in BARI GOM 26 were the highest in Treatment T5 (0.67 gm/plant) and the lowest in Treatment T1 (control) (0.32 gm/plant) respectively, followed by gradual increase in Treatments T2 (0.42 gm/plant), T3 (0.56 gm/plant) and Treatment T4 (0.61 gm/plant). Again, for BARI GOM 25 total root biomass were the highest in TreatmentT5 (0.65 gm/plant) and the lowest in Treatment T1 (0.30 gm/plant) respectively, followed by gradual increases in Treatments T2 (0.40 gm/plant), T3 (0.55 gm/plant) and Treatment T4 (0.60 gm/plant).

Similar to plant height and shoot biomass, root biomass was also significantly ($P \leq 0.001$) affected among all the various P application on wheat plant.

Table 4.4: Root biomass, shoot biomass and root/shoot ratio of two wheat varieties across different P levels

Varieties	Treatments	P rates (mg/kg)	Biomass Production (gm/pot)		Root-shoot ratio
			Shoot	Root	
BARI GOM 25	T1	0	0.45	0.30	0.66
	T2	30	0.55	0.40	0.73
	T3	60	0.76	0.55	0.73
	T4	90	0.80	0.60	0.75
	T5	120	0.85	0.66	0.77
BARI GOM 26	T1	0	0.47	0.32	0.69
	T2	30	0.57	0.42	0.73
	T3	60	0.76	0.56	0.74
	T4	90	0.81	0.61	0.75
	T5	120	0.87	0.67	0.78

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 4.4). Comparison of different treatment root: shoot ratio showed an increase with the increasing P application in both varieties of BARI released wheat plants. In the same line, Bhadoria *et al.*, (2002) reported that, phosphate shortage increased root/shoot ratio of wheat, because shoot growth was more reduced than development. Similar results were observed in lettuce (Buso and Biss, 1988), and maize (Gaume *et al.*, 2001).

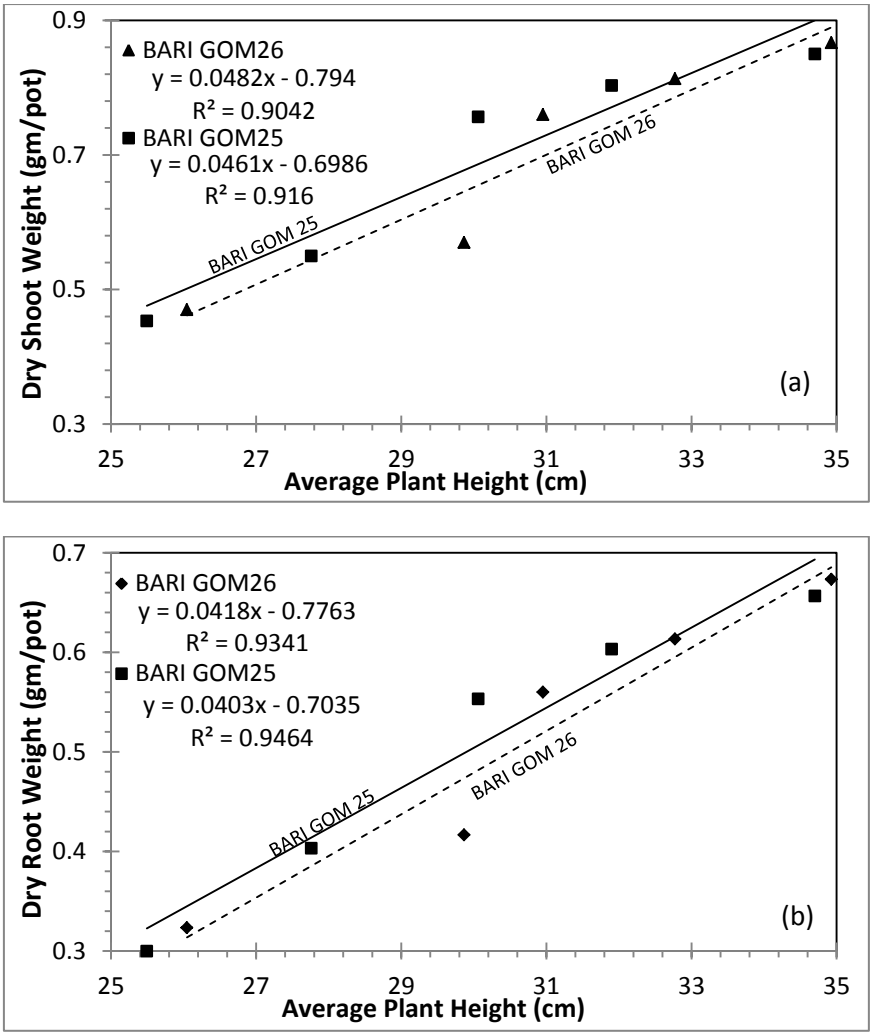

Figure 4-9: Relationship between (a) shoot dry weight and average plant height; (b) root dry weight and average plant height of wheat plant

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= 0.92, $p < 0.05$) between plant height and shoot biomass under elevated P supply indicated that plant development enhances with the application of P in soil (Figure 4-9). Similarly, a significant correlation (R^2 = 0.95, p<0.05) between plant height and root biomass under elevated P application was observed. The increase in plant growth is largely due to increase absorption of

nutrients from soil solution (Son and Smith, 1988). However, the elevated P plays significant role in the growth of the wheat plant.

4.6 Conclusion

This study reveals that the elevated P taken a significant part in the development of the wheat plant in acidic soil. These findings indicate 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 is found in various experiments. Further extensive research and keen observation of trials are necessary to determine the effects of high phosphorus application. Our next research step is to analyses the plants and soils in order to determine the actual soil phosphorus availability.

Chapter 5

Effect of elevated phosphorus on growth response to wheat plant that grown in an alkaline soil

Chapter 5

5. Effect of elevated phosphorus on growth response to wheat plant that grown in an alkaline soil

5.1 Introduction

Phosphorus is a macronutrient that plays a numerous important roles in plants. It is involved in several key plant functions including energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant and transfer of genetic characteristics from generation after generation (Pasek 2008). The P availability is one of the major growth limiting factors in many ecosystems around the world (Barber *et al.*, 1963). Large amounts of P fertilizers are generally required for sustainable crop production on variable charge soils because of low P availability to plants (Barrow, 1986; Lin, 1995). In sandy soils of arid area of southern east Algeria, P is influenced by various factors such as alkaline (pH>7) soil conditions and the high $CaCO_3$ content (>3%). Regular applications of ordinary superphosphate to sandy soils in laboratory leaching experiments led to a buildup of acid extractable inorganic P even though more than 80% of P in the fertilizer was lost during the leaching phase following application (Ritchie and Weaver, 1993).

Due to these interactions, nearly 80% of applied P as fertilizers may be fixed in the soil (Barrow, 1980; Holford, 1997). According to Raghothama *et al.*, (2005) and Rahim *et al.*, (2007), P deficiency is very common in alkaline calcareous soils. The amount of soil P removed by crops need to be replenished through the application of fertilizer P and manure to maintain soil P balance (Saleque *et al.*, 2006).

Our previous study showed that P use efficiency by recently BARI released wheat plant that grown in an acidic soil. However, no study was undertaken about P use efficiency in an alkaline soil. Therefore, this study aims with the following objectives: to understand how elevated P behaves in an alkaline soil, to determine P uptake by recently BARI released wheat plant under alkaline condition, evaluate growth response of recently BARI released wheat varieties under elevated P applied condition. This study hypothesized that elevated P will help to utilized more P by the wheat plant even in alkaline condition.

5.2 Materials and Methods

5.2.1 Soil and plant

In this study, alkaline soil was used as experimental soil. The initial soil pH was 7.9 in water. The basic properties of soil are out-lined in Table 5.1. The BARI GOM 25 and BARI GOM 26 wheat varieties were used as a testing plant.

Table 5.1: Properties of soils used in this experiment

Soil pH	Total N (%)	Available P (ppm)	Exchangeable K (Cmol/Kg)	Available S (ppm)	Available Zn (ppm)	Organic matter (%)
7.9	0.03	14.3	0.21	5.6	11.55	0.55

5.2.2 Experimental design

The completely Randomized Design (CRD) was adopted. The CRD experiment consisted of five levels of Phosphorus (P)–0, 30, 60, 90 and 120 mg/kg P and two wheat varieties- BARI GOM 25 and BARI GOM 26 with three replications. The KH_2PO_4 chemical was used as a P source. To avoid the interactions between soil nutrients and added P, no basal nutrients were added in this experiment. 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.

5.2.3 Experiment procedure

The soil was incubated at 30°C for 7 days, then KH_2PO_4 as per P doses were 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 & Agricultural Extension, Rajshahi University, Rajshahi, Bangladesh.

5.2.4 Seed germination and plant sowing

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 petri dishes in dark at 25°C for 70h. Five holes (1.0 cm deep and 1.0 cm wide) were made in the soil in each plastic cup containing 200g pre-incubated soil. Keeping the radicals in down ward direction, five pre-germinated seeds of BARI GOM 25 and BARI GOM 26 were

placed carefully in those holes in each cup and then the seeds were gently covered with the same treated soil. The BARI GOM 25 and BARI GOM 26 were placed in separate cups. After sowing, each cup was covered with filter paper for first two days to avoid disturbance of top soil. Deionised (DI) water was sprayed on the filter paper to keep the soil moist. To maintain the required soil moisture content 20 ml DI water was added every day to each cup during the growing period of wheat plants and watering was stopped at 3 days before harvesting.

5.2.5 Plant growth condition

Plants were grown in open air under 10h dark and 14h light conditions. The variations of day and night temperature were 20-30°C and 15-20°C respectively. All the cups were re-randomised in their position on alternate days during the growing period of wheat seedlings, to minimize the positional effects.

5.2.6 Plant harvesting

Plants were harvested after 28 days of sowing. Whole plants with roots and surrounding soil were removed from each cup by gentle agitating to provide minimum disturbance to the roots and shoots. Intact plants were then lifted up gently from the soil and shaken lightly to remove the bulk soil and then washed to remove the adhered soil from roots. Collected bulk soil was air-dried and stored in a controlled room temperature (25°C) until analysis. Shoots and roots were separated. Shoots were oven dried at 70°C for 3 days and stored in an oven for analysis. The harvested roots were

washed with DI water and oven-dried at 70°C temperature for 3 days and stored for analysis.

5.3 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 percloric acids (4:1), the concentration of P in root and shoot materials were determined using the vanadomolybdate method. Colorimetric method for the determination of phosphorous 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 DI water. The absorbance reading was used at 470 nm (Iqbal *et al.*, 2010).

5.3.1 Phosphorus use efficiency calculation

Phosphorus use efficiency, PUE was calculated using the formulae as described by Fageria *et al.*, (1997).

$$PUE\% = \frac{Total\ P\ uptake\ \left(\frac{mg}{kg}\right) in\ Treatment\ pot - Total\ P\ uptake\ \left(\frac{mg}{kg}\right) in\ control\ pot}{P\ dose\ applied\ (\frac{mg}{kg})} \times 100$$

Total P uptake (mg/kg) = P uptake by root (mg/kg) + P uptake by shoot (mg/kg)

5.3.2 Measurements of soil physical and chemical properties

Soil textural analysis was conducted by using an abbreviated version of the international pipette method. Clay content was determined by 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 experiment 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 Asija, 1956). The distillate was collected in 20 ml of 2% boric acid solution with methylred and bromocresol green indicator and titrated with 0.02 N sulphuric acid (H_2SO_4) (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 NH_4OAC and determined by an atomic absorption spectrometer (Biswas *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 $NaHCO_3$ 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).

5.3.3 Statistical analysis

Shoot and root parameters were analysed by two-way ANOVA (Treatment × Varieties), 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 (Lawes Agricultural Trust, UK).

5.4 Results

5.4.1 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 5-1. The results showed for the variety BARI GOM 25 that the maximum plant height (31.9 mm) was recorded in Treatment T5 (120 mg/kg P), while it was minimum (21.96 mm) in Treatment T1 (control). Again, the results showed for the variety BARI GOM 26 that the maximum plant height (32.8 mm) was recorded in Treatment T5 (120 mg/kg P), while it was minimum (22.97 mm) in Treatment T1 (control). Plant height was significantly ($P \leq 0.001$) affected among all the various P applications and variety of wheat plants. It also increased with the increasing level of phosphorus application. Hence, among the low level of various phosphorous application, Phosphate had more plant height while at high level P resulted in maximum plant height.

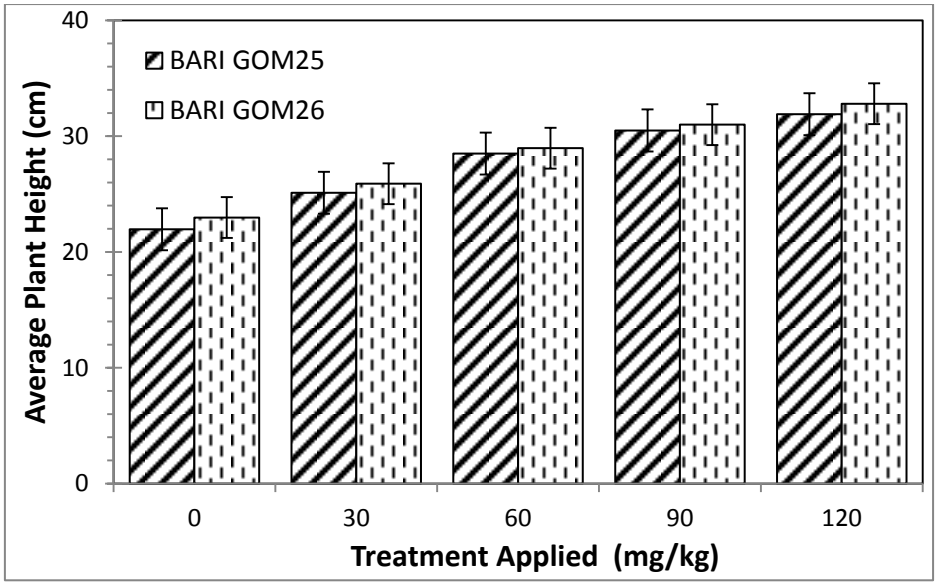

Figure 5-1: Effect of P application on average plant height of the wheat seedlings that was grown in various level of P for 28 days.

All the plant growth and P-uptake parameters were highly significant ($P \leq 0.001$) under P levels (Table 5.2). Similarly, significant differences among varieties were observed in relation to all the growth and P-uptake parameters.

Table 5.2: Significance levels for the main and interactive effect of P and wheat 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)	***	n.s.	**	n.s.	**
T×V	***	n.s.	-	n.s.	-

Where n.s., ** and *** represent probability of > 0.05, ≤ 0.01 and ≤ 0.001, respectively.'-' indicates no data available.

5.4.2 Shoot dry weight

Like plant height, the shoot biomass showed similar trend under different P applications. The results showed for the variety BARI GOM 25 that the maximum shoot biomass (0.81 gm/plant) was recorded in Treatment T5 (120 gm/kg P), while it was minimum (0.38 gm/plant) in Treatment T1 (control). Again, the results showed for the variety of BARI GOM 26 that the maximum shoot biomass (0.83 gm/plant) was recorded in Treatment T5 (120 gm/kg P), while it was minimum (0.40 gm/plant) in Treatment T1 (control). 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 ($P > 0.05$) differed between varieties of wheat plant. The shoot biomass also increased with the increased level of P application (Figure 5-2).

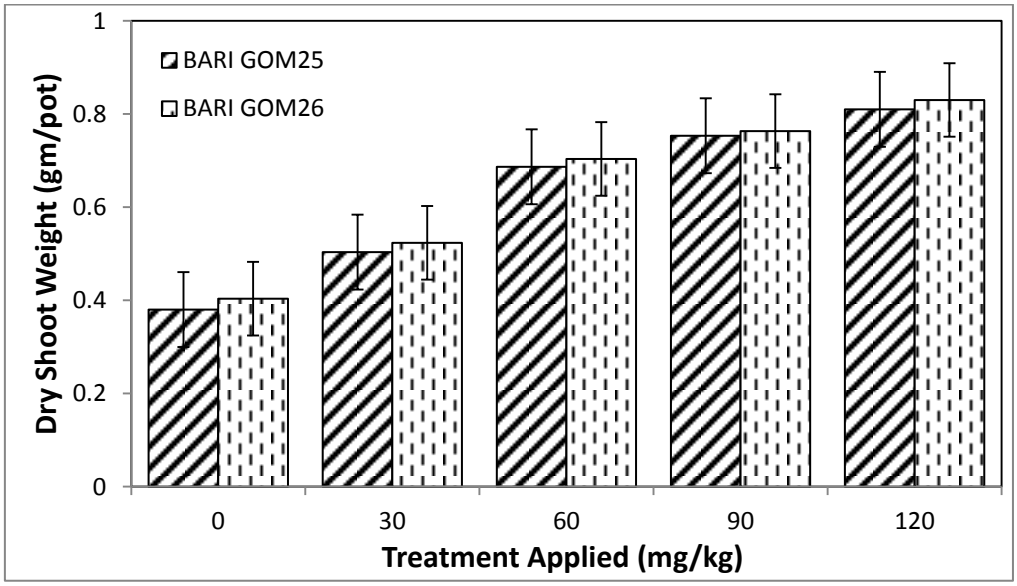

Figure 5-2: Effect of P application on dry shoot weight of the wheat seedlings that was grown in various level of P for 28 days.

5.4.3 Root dry weight

Total root biomass varied among the treatments. Total root biomass of BARI GOM 26 in Treatment T5 was highest (0.63gm/plant) and lowest in Treatment T1 (0.26 gm/plant), followed by gradual increase in the Treatments T2 (0.36 gm/plant), T3 (0.50 gm/plant) and Treatment T4 (0.56 gm/plant) (Figure 5-3). Again, for BARI GOM 25, the total root biomass was highest in Treatment T5 (0.60 gm/plant) and lowest in Treatment T1 (0.25 gm/plant), followed by gradual increase in Treatments T2 (0.35 gm/plant), T3 (0.48 gm/plant) and Treatment T4 (0.55 gm/plant). Similar to shoot dry weight, root biomass was significantly ($P \leq 0.001$) affected among all the various P application on wheat plant. But, the root biomass did not significantly ($P>0.05$) differ among varieties of wheat plants. The root biomass also increased with the increasing level of phosphorus application (Figure 5-3).

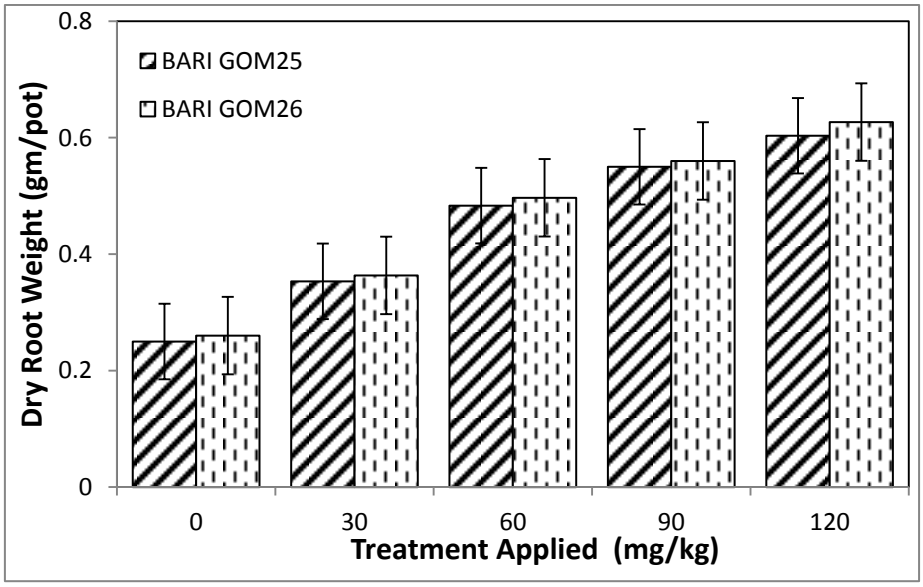

Figure 5-3: Effect of P application on dry shoot weight of the wheat seedlings that was grown in various level of P for 28 days.

5.4.4 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 application on wheat plant. Total shoot P concentration in BARI GOM 26 in Treatment T5 was the highest (4.49 gm/kg) and in Treatment T1 was the lowest (0.43 gm/kg), followed by gradual increase in Treatments T2 (1.29 gm/kg), T3 (3.72 gm/kg) and T4 (4.32 gm/kg) (Figure 5-4). Again, for BARI GOM 25 total shoot P concentration in Treatment T5 and T1 were the highest (4.41 gm/kg) and the lowest (0.41 gm/kg) respectively, followed by gradual increase in Treatments T2 (1.21 gm/kg), T3 (3.61 gm/kg) and T4 (4.23 gm/kg). The shoot P concentration of BARI GOM 25 and BARI GOM 26 were dependent on the treatments. However, the two varieties had similar responses on shoot P concentration in the different treatments (Figure 5-4).

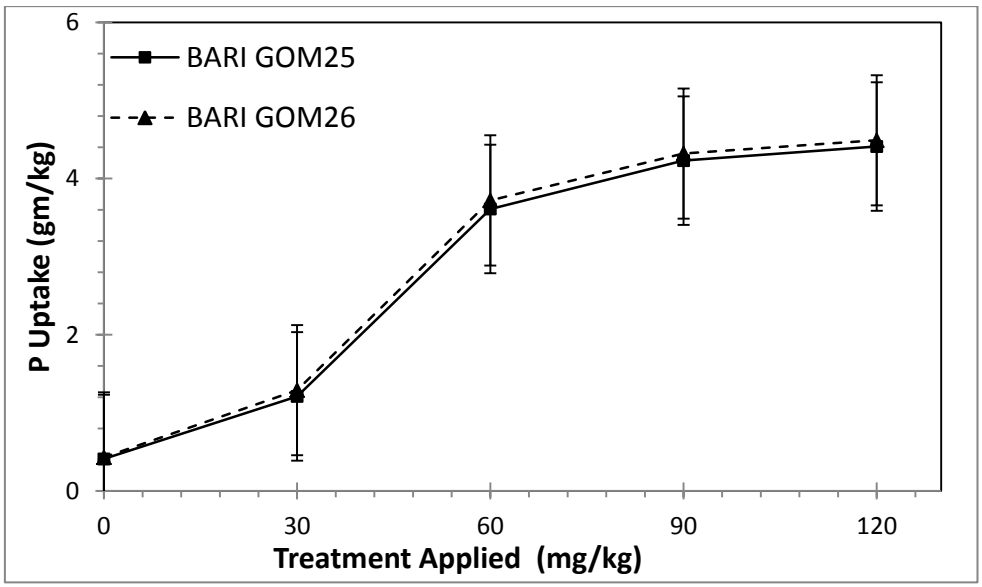

Figure 5-4: Effect of P application on P uptake of wheat shoot in various level of P for 28 days.

5.4.5 Root P concentration

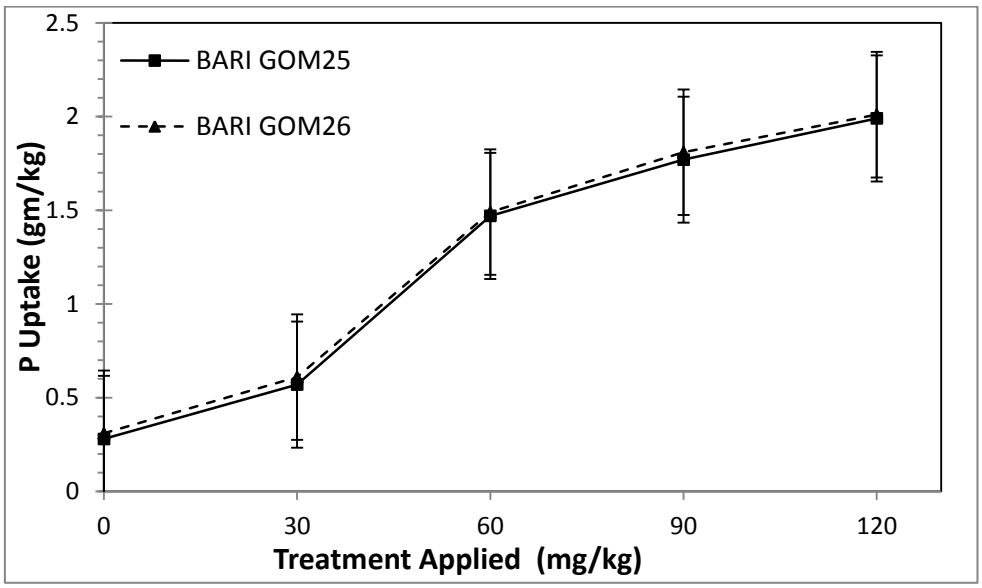

Figure 5-5: Effect of P application on P uptake of wheat root in various level of P for 28 days.

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 in Treatment T5 and T1 were the highest (2.01 gm/kg) and the lowest (0.31 gm/kg) respectively, followed by gradual increase in Treatments T2 (0.61 gm/kg), T3 (1.49 gm/kg) and T4 (1.81 gm/kg) (Figure 5-5). Again, for BARI GOM 25 total root P concentration in Treatment T5 and T1 were the highest (1.99 gm/kg) and the lowest (0.28 gm/kg), followed by gradual increase in Treatments T2 (0.57 gm/kg), T3 (1.47 gm/kg) and T4 (1.77 gm/kg). The root P concentration of BARI GOM 25 and BARI GOM 26 were dependent on the treatments. However, the two varieties had similar responses on root P concentration in the different treatments (Figure 5-5).

5.4.6 Total P uptake and P distribution

In both varieties of BARI GOM 25 and BARI GOM 26 were found similar trend in total P uptake. The total P uptake by wheat plant was significantly high in Treatment T5 from other treatments in both varieties. However, total P uptake was about nine times greater in Treatment T5 than control Treatment T1. The total P uptake was greater in BARI GOM 26 than BARI GOM 25 in all treatments. Total P uptake in BARI GOM 26 of Treatment T5 and T1 were the highest (6.50 gm/kg) and the lowest (0.74 gm/kg), followed by gradual increase in Treatments T2 (1.90 gm/kg), T3 (5.21 gm/kg) and Treatment T4 (6.13 gm/kg) (Figure 5-6). Again, for BARI GOM 25 total P uptake in Treatment T5 and T1 were the highest (6.40 gm/kg) and the lowest (0.69 gm/kg), followed by gradual increase in Treatments T2 (1.78 gm/kg), T3 (5.08 gm/kg) and Treatment T4 (6.0 gm/kg) (Figure 5-6).

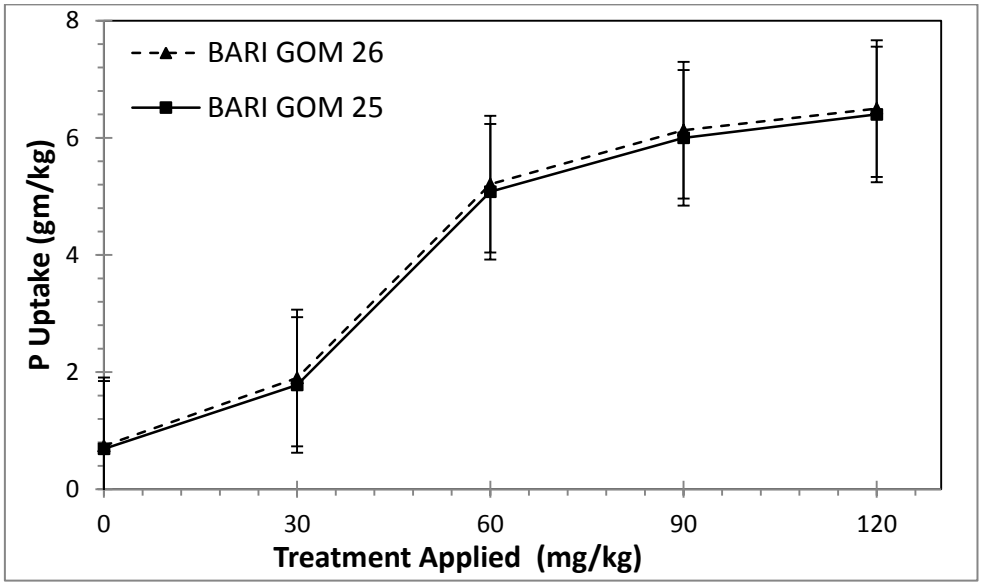

Figure 5-6: Effect of P application on P uptake of wheat plant in various level of P for 28 days.

5.4.7 P use efficiency in wheat plant

Data on P use efficiency (PUE) in wheat are given in Table 5.3. The PUE was calculated in terms of P uptake per unit of P application. The results revealed that lower PUE was seen at higher P rates. The maximum PUE of 7.32 % was observed at 60 mg/kg P rate for variety BARI GOM 25 and it decreased significantly at higher P rates. Similarly, the maximum PUE of 7.53 % was observed at 60 mg/kg P rate for variety BARI GOM 26 and it decreased significantly at higher P rates. Again, variety significantly affected the PUE and the minimum of 3.63 % was obtained with 30 mg/kg P application for variety BARI GOM 25 and 4.03 % was obtained with 30 mg/kg P application for variety BARI GOM 26. Results are in conformity with those of Rahim *et al.*, (2010) who concluded that wheat growth increased significantly with the use of P. However, it is clear from Table 5.3 that P application through band placement significantly enhanced the PUE with that of broad casting.

Table 5.3: P concentration in root, P concentration in shoot and P use efficiency of two wheat varieties across different P levels

Variety	Treatment	P rate (mg/kg)	P in Shoot (gm/kg)	P in Root (gm/kg)	Total P uptake (gm/kg)	P use efficiency, PUE (%)
BARI GOM 25	T1	0	0.41	0.28	0.69	0.00
	T2	30	1.21	0.57	1.78	3.63
	T3	60	3.61	1.47	5.08	7.32
	T4	90	4.23	1.77	6.00	5.90
	T5	120	4.41	1.99	6.40	4.76
BARI GOM 26	T1	0	0.43	0.31	0.74	0.00
	T2	30	1.29	0.61	1.90	4.03
	T3	60	3.72	1.49	5.21	7.53
	T4	90	4.32	1.81	6.13	6.04
	T5	120	4.49	2.01	6.50	4.84

5.5 Discussion

5.5.1 Effect of elevated phosphorus application on alkaline soil

In general, plants grow better when partially soluble phosphate is applied in comparison to the soluble P source. In this respect, Rubio *et al.*, 2003 has published a details discussion on the effect of the pH change over P solubility in soil and its availability. Soil pH influences the charge of the P species in solution more than the charge of the adsorbing particles in soils. When pH rises, an increase in the negative charge, $H_2PO_4^-$ is produced thereby decreasing P adsorption; but, at the same time, an increase in divalent HPO_4^{2-} ion is produced which has a much greater affinity for reactive soil surfaces thereby increasing P adsorption (Schachtman *et al.*, 1998). Plant roots take up P from the soil solution principally as monovalent orthophosphate $H_2PO_4^-$ ions and to a lesser extent as divalent orthophosphate, HPO_4^{2-} ions, except in calcareous and saline soils. Plant roots can absorb P from soil solutions having very low P concentrations (Loneragan and Asher, 1967), in which case P uptake is against a very steep P concentration gradient. This is because the P content of root cells and xylem sap is 100–1000 times larger than that of the soil solution (Mengel and Kirkby, 1987). The transport of P across the cell membrane varies between plant species. Cultivars within the same species can differ in their capacity for active P uptake, and these differences are probably largely genetically controlled. The study was conducted in alkaline soil and P doses were applied directly to the soil. The maximum P use efficiency of 7.32 % was observed at 60 mg/kg P rate for variety BARI GOM 25 and it decreased significantly at higher P rates. Similarly, the maximum P use efficiency of 7.53 % was observed at 60 mg/kg P rate for variety BARI GOM 26 and

it decreased significantly at higher P rates. Results indicate that plant growth increased significantly with the use of P. However, it is clear from Figure 5-7 that P application through band placement significantly enhanced the PUE with that of broad casting.

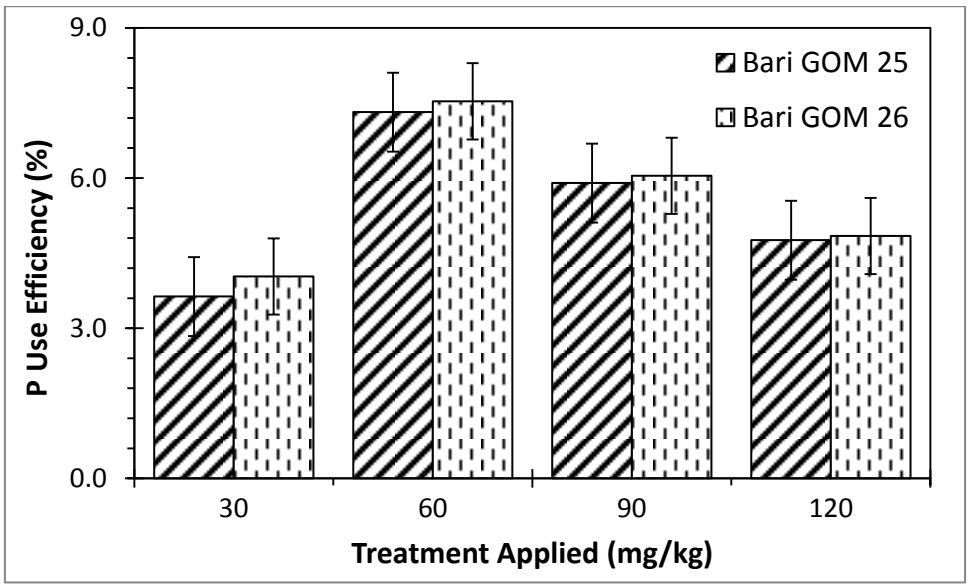

Figure 5-7: P use efficiency of two wheat varieties across different P levels

5.5.2 Phosphorus uptake efficiency by wheat plant under alkaline condition

After harvesting, the shoot P uptake amounts of the different treatments were compared. At low level of P supply, shoot P uptake was significantly increased in comparison with the controlled treatment (Figure 5-1). At high levels of available P, there was no significant difference between all treatments. Similar results were observed in different genotypes of wheat (Fageria and Baligar (1999); Ahmad *et al.*, (2013); Hu *et al.*, (2014)). Total shoot P concentration in BARI GOM 26 of Treatment T2 increased 67% (1.29 gm/kg) in comparison with the controlled Treatment T1 (0.43 gm/kg). Similar trend was found in T3 65% (3.72 gm/kg) in

comparison of T2. But, at high levels of available P, in Treatment T4 and Treatment T5 the shoot P concentrations were increased 14% and 4 % respectively. Again, for BARI GOM 25 total soot P concentration in Treatment T2 increased 66% (1.21 gm/kg) in comparison with the controlled Treatment T1 (0.41 gm/kg). Similar trend was found in T3 66% (3.61 gm/kg) in comparison of T2. But, at high levels of available P, in treatment T4 and Treatment T5 the shoot P concentrations were increased 15% and 4 % respectively. This is in line with the suggestion of Cavagnaro *et al.*, (2003) that, P uptake by plant shoot was significantly high at low P concentration.

During collection, the root P uptake measures of the diverse treatments were analyzed. At low level of P supply, root P uptake, on examination, was found to increase primarily with respect to controlled treatment (Figure 1). At elevated amounts of accessible P, there was no noteworthy contrast among all treatments. Comparative results were shown in different genotypes of wheat (Hu *et al.*, (2014)). Aggregate root P fixation in BARI GOM 26 of Treatment T2 was increased 49% (0.61 gm/kg) in correlation with the controlled Treatment T1 (0.31 gm/kg). Comparable pattern was found in T3 59% (1.41 gm/kg) in correlation with treatment T2. At high levels of accessible P, in Treatment T4 and Treatment T5 the root P fixation were 18% and 10 % increased respectively. Again, for BARI GOM 25 aggregate root P concentration in Treatment T2 increased 51% (0.57 gm/kg) on examination with respect to the controlled Treatment T1 (0.28 gm/kg). Comparative pattern was found in T3 61% (1.47 gm/kg) on examination of Treatment T2. However, at high levels of accessible P, in Treatment T4 and Treatment T5 the root P fixations increased 17% and 11 % respectively. This is in accordance with the

recommendation of Cavagnaro *et al.*, (2003), that P uptake by plant root was primarily high at low P concentration.

The plant P uptake measure of the various treatments was investigated. Similar to the shoot and root phosphorus uptake pattern, total plant P uptake results were compared with different treatments. Total plant P uptake in BARI GOM 26 of Treatment T2 increased 61% (1.90 gm/kg) in relationship with the controlled Treatment T1 (0.74 gm/kg). Similar example was found in T3 64% (5.21 gm/kg) in connection of treatment T2. At high levels of accessible P, in treatment T4 and Treatment T5 the plant P uptake were increased 15% and 6 % independently. Again, for BARI GOM 25 total plant P uptake in Treatment T2 increased 61% (1.78 gm/kg) in examination with the controlled Treatment T1 (0.69 gm/kg). Similar example was found in T3 65% (5.08 gm/kg) in examination of Treatment T2. On the other hand, at large levels of available P, in Treatment T4 and Treatment T5 the plant P uptake were increased 15% and 6 % independently. Thus, P uptake by wheat plant was on a very basic level, high at low P fixation.

The relationship between shoot P uptake and average plant height was analyzed to investigate the P taken up by plants. A significant correlation (R^2= 0.96, $p < 0.05$) between plant height and shoot P uptake under elevated P supply indicates that plant development enhances with the application of P in soil (Figure 5-8). Similarly, a significant correlation (R^2 = 0.97, p <0.05) between plant height and root P uptake under elevated P application was observed. The relationship indicates that increase of P uptake plant height increases with elevated P application which makes increasing absorption of nutrients from soil solution.

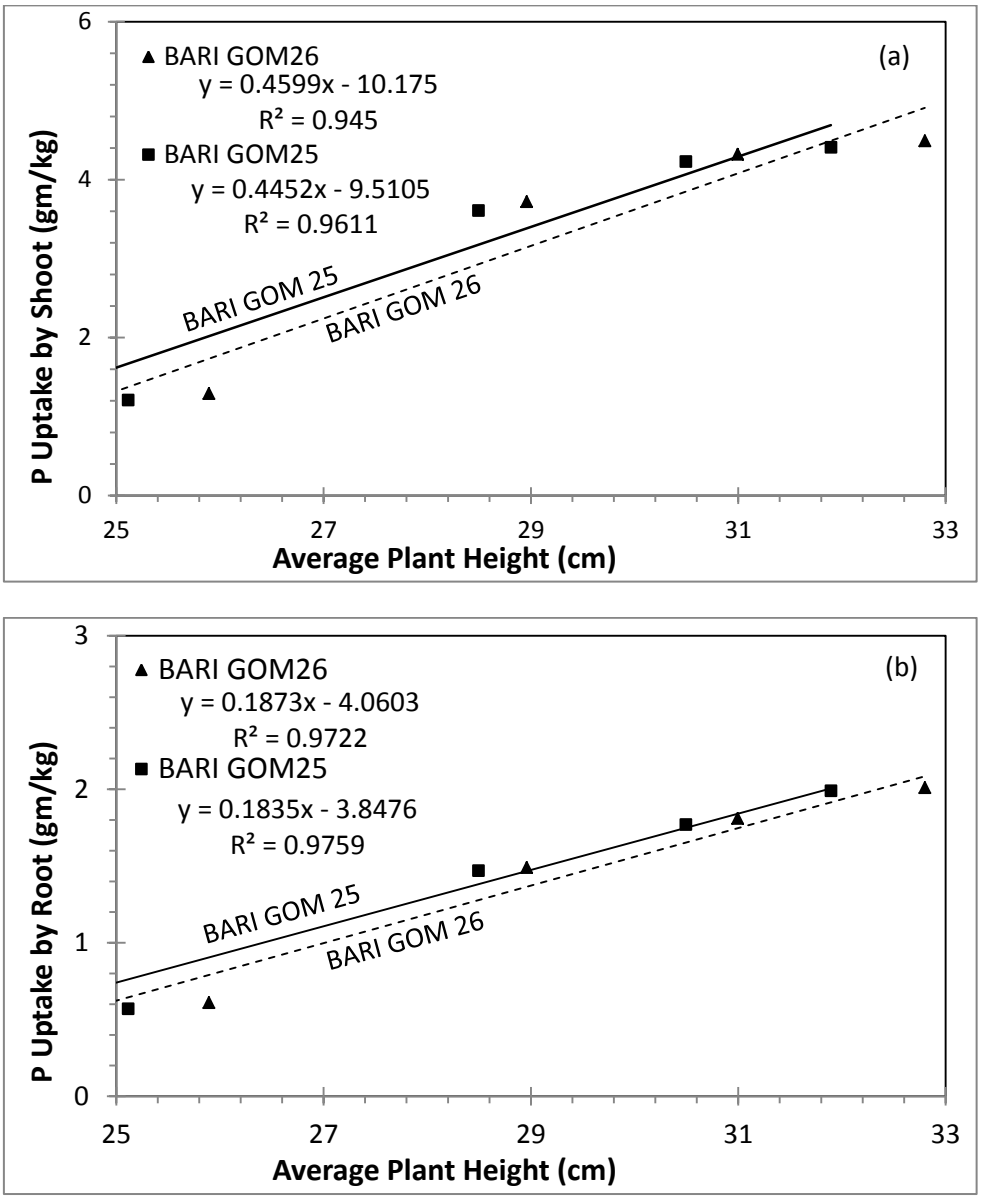

Figure 5-8: Relationship between (a) P uptake by shoot and average plant height; (b) P uptake by root and average plant height of wheat plant

5.5.3 Growth response of wheat plant for elevated P application in alkaline soil

In find out the 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 done. The highly significant Treatment (T)

interaction for shoot growth ($P \leq 0.001$) in this study indicates that the shoot growth responses of BARI GOM 25 and BARI GOM 26 seedlings were dependent on the level of added P. In all treatments, there were no significant differences between BARI GOM 25 and BARI GOM 26 seedlings for any growth measurement. The results showed for the variety BARI GOM 25 that the maximum plant height (31.9 mm) was recorded in treatment T5 (120 mg/kg P), while it was minimum (21.96 mm) in treatment T1 (control). Again, the results showed for the variety BARI GOM 26 that the maximum plant height (32.8 mm) was recorded in treatment T5 (120 mg/kg P), while it was minimum (22.97 mm) in treatment T1 (control). Thus, plant height significantly ($P \leq 0.001$) affected among all the various P applications and variety of wheat plants.

The shoot biomass also significantly ($P \leq 0.001$) affected among all the various P applications on wheat plants. The experimental results indicated for the variety BARI GOM 25 that the maximum shoot biomass (0.81 gm/plant) was recorded in Treatment T5 (120 gm/kg P), while it was minimum (0.38 gm/plant) in Treatment T1 (control). Again, the results indicated for the variety BARI GOM 26 that the maximum shoot biomass (0.83 gm/plant) was recorded in Treatment T5 (120 gm/kg P), while it was minimum (0.40 gm/plant) in Treatment T1 (control).

Investigation on another growth parameter, total root biomass varied among the treatments. Total root biomass in BARI GOM 26 of Treatment T5 was highest (0.63 gm/plant), followed by gradually increase in Treatments T2 (0.36 gm/plant), T3 (0.50 gm/plant) and Treatment T4 (0.56 gm/plant) and in Treatment T1 (0.26 gm/plant) was lowest. Again, for BARI GOM 25 total root biomass in Treatment T5 was highest (0.60 gm/plant), followed by gradually increase in Treatments T2 (0.35 gm/plant), T3 (0.48 gm/plant) and Treatment T4 (0.55 gm/plant) and in Treatment T1 (0.25

gm/plant) was lowest. Similar to plant height and shoot biomass, root biomass was also significantly ($P \leq 0.001$) affected among all the various P application on wheat plant.

Table 5.4: Root biomass, shoot biomass and root/shoot ratio of two wheat varieties across different P levels

Variety	Treatment	P rate (mg/kg)	Biomass Production (gm/pot)		Root-shoot ratio
			Shoot	Root	
BARI GOM 25	T1	0	0.38	0.25	0.66
	T2	30	0.50	0.35	0.70
	T3	60	0.69	0.48	0.70
	T4	90	0.75	0.55	0.73
	T5	120	0.81	0.60	0.74
BARI GOM 26	T1	0	0.40	0.26	0.64
	T2	30	0.52	0.36	0.69
	T3	60	0.70	0.50	0.71
	T4	90	0.76	0.56	0.73
	T5	120	0.83	0.63	0.76

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 5.4). Comparison of different treatment shows that root: shoot ration increased with the increasing P application in both variety of BARI released wheat plant. On the same line, Bhadoria *et al.*, (2002) reported that, phosphate shortage increased root/shoot ratio of wheat, because shoot growth was more reduced than development. Similar results were observed in lettuce (Buso and Biss, 1988), and maize (Gaume *et al.*, 2001).

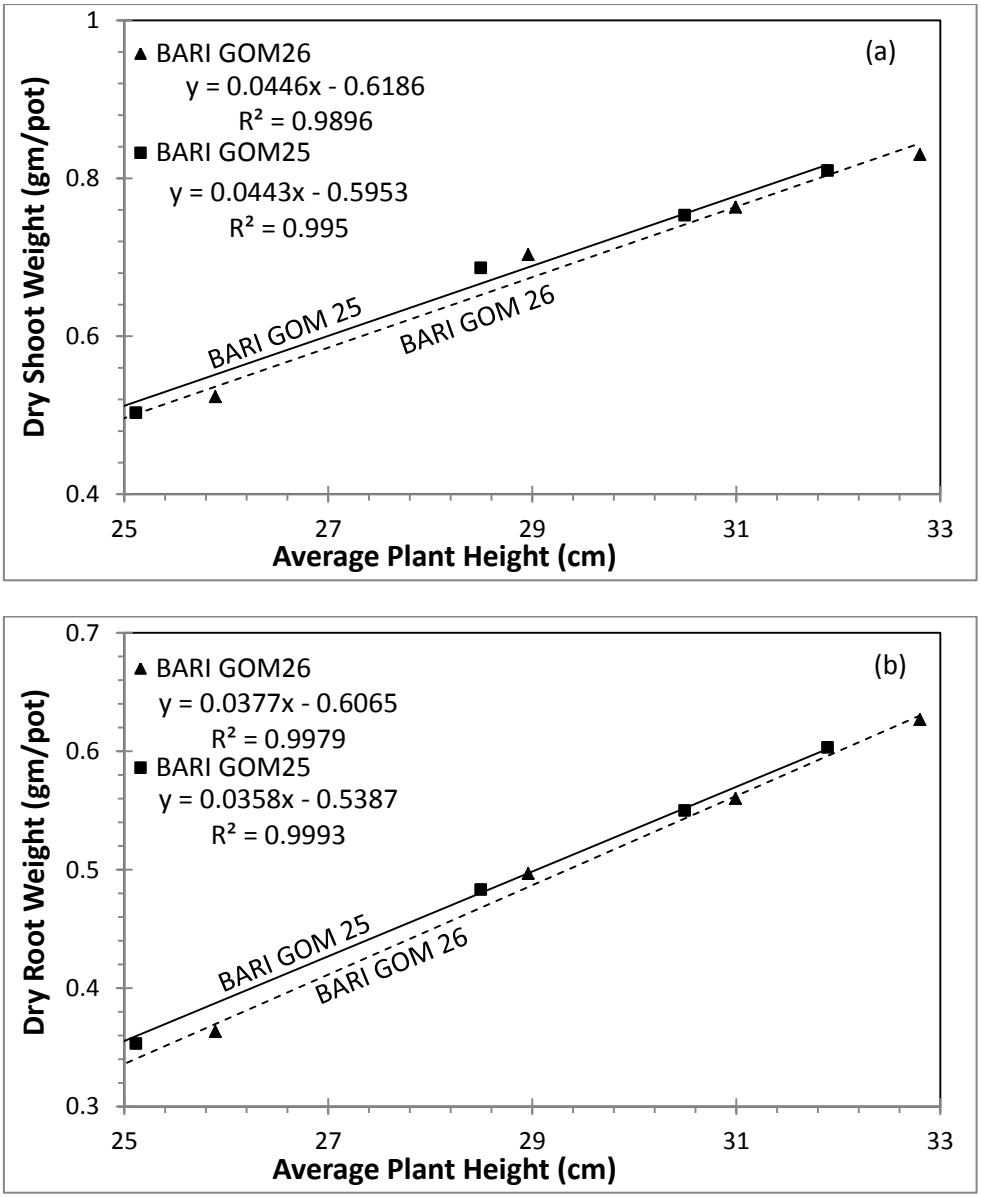

Figure 5-9: Relationship between (a) shoot dry weight and average plant height; (b) root dry weight and average plant height of wheat plant

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= 0.99, $p < 0.05$) between plant height and shoot biomass under elevated P supply indicated that plant development enhances with the application of P in soil (Figure 5-9). Similarly, a significant correlation (R^2 = 0.99, p <0.05) between plant height and root biomass under elevated P application was

observed. The increase in plant growth is largely due to increasing absorption of nutrients from soil solution (Son and Smith, 1988). However, the elevated P plays a significant role in the growth of the wheat plant.

5.6 Conclusion

This study exhibits that the elevated P takes a very significant part in the development of the wheat plant in alkaline soil. The findings indicates that the added soluble P increases the absorption of nutrients from the soil solution. The added P is efficient both for shoot development and root growth. No varietal difference is found within this experiment. Further research and observation of trial are necessary to find out more information about the effects of elevated phosphorus application. Further study will be conducted in split-root system under acidic and alkaline soil combination with the growth response of recently released BARI wheat varieties from the following chapters.

Chapter 6

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

Chapter 6

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

6.1 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 the plants in orthophosphate ($H_2PO_4^-$ and HPO_4^{2-}) forms (Hinsinger, 2001). Phosphorus is a structural component of many co-enzymes, phospho-proteins, phospholipids (Ozanne, 1980) and part of the genetic memory "DNA" 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 for crop production in acidic soils. 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 (Norman and Hemwall, 1957). This means that inorganic P is often a limiting factor in plant growth and development. 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

symbiosis with arbuscular mycorrhizas or ectomycorrhizas (Péret *et al.*, 2011; Smith *et al.*, 2011). Phosphorus is readily translocated within the plants, moving from older to younger tissues as the plant forms cells and develops roots, stems and leaves. 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. To understand 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 and to quantify how translocated phosphorus effects on wheat plant within split-root system under P efficient condition.

6.2 Materials and Methods

6.2.1 Soil and Plant

Acidic soil collected from Thakurgaon district, Bangladesh, was used as experimental soil. The initial soil pH was 5.2. The basic properties of soil are out-lined in Table 6.1. The BARI GOM 25 and BARI GOM 26 wheat varieties were used as testing plants.

Table 6.1: Properties of soils used in this experiment

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

6.2.2 Experimental design

The split-root experiment was conducted with the treatments described in Table 6.2. The BARI GOM 25 and BARI GOM 26 were compared. The treatments were replicated three times. The KH_2PO_4 chemical was used as a P source. 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 30°C for 7 days 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 & Agricultural Extension, Rajshahi University, Rajshahi.

Table 6.2: Split-root system with different treatments

Treatment	Symbols	Treatment symbols		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

6.2.3 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.

6.2.4 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 was continued for 28 days.

6.2.5 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-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. A 20 ml water was added to each compartment every day and watering was stopped 3 days before harvesting.

6.2.6 Harvesting

The experimental plants were harvested 28 days after transplanting. The shoots were cut 0.5 cm above the base part of the stem uniformly. Then the roots were cut 0.5 cm below the base part and separated carefully into two halves as previously marked. Soils from two root halves removed carefully so that the roots could not be toned 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 over dried at the same temp for the same time. After drying, the root and shoot samples were weighed and stored for analytical experiments.

6.3 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 percloric acids (4: 1), the concentration of P in root and shoot materials were determined using the vanadomolybdate method after digestion in a mixture of concentrated nitric and perchloric acids (4:1). Colorimetric method for the determination of phosphorous 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 DI water. The absorbance reading was used at 470 nm (Iqbal *et al.*, 2010).

6.3.1 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 H_2O_2 to remove organic matter (Gee and Bauder, 1986). The pH of the soil was determined before incubation in deionized 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 Asija, 1956). The distillate was collected in 20 ml of 2% boric acid solution with methylred and bromocresol green indicator and titrated with 0.02 N sulphuric acid (H_2SO_4) (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 NH_4OAC and determined by an atomic absorption spectrometer (Biswas *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 $NaHCO_3$ 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).

6.3.2 Statistical analysis

Shoot and root parameters were analyzed by two-way ANOVA (Treatment × Variety), 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 (Lawes Agricultural Trust, UK).

6.4 Results

All the plant growth and P-uptake parameters were highly significant ($P \leq 0.001$) under P levels (Table 6.3). Similarly, significant differences among varieties were observed in relation to all the growth and P-uptake parameters.

Table 6.3: Significance levels for the main and interactive effect of P and wheat 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)	***	n.s.	***	*	*
Compartment (C)	-	-	-	***	***
T×V	**	n.s.	***	n.s.	n.s.
T×C	-	-	-	***	***
C×V	-	-	-	n.s.	n.s.
T×V×C	-	-	-	n.s.	n.s.

Where n.s., ** and *** represent probability of > 0.05, ≤ 0.01 and ≤ 0.001, respectively, and '-' (dash) indicates no data available.

6.4.1 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 6-1. The results showed for the variety BARI GOM 25 that the maximum plant height (34.75 mm) was recorded in treatment C (50P/200P mg/kg), while it was minimum (28.79 mm) in treatment A (control). Again, the results showed for the variety BARI GOM 26 that the maximum plant height (35.89 mm) was recorded in treatment C (50P/200P mg/kg), while it was minimum (30.73 mm) in treatment A (control). Plant height was significantly ($P \leq 0.001$) affected among all the various P applications and variety of wheat plants. It also increased with the increasing level of phosphorus application. Hence, among low level of various phosphorous application, Phosphate had the gradual increasing effect on plant height with increasing P applications, while at high level P resulted in maximum plant height.

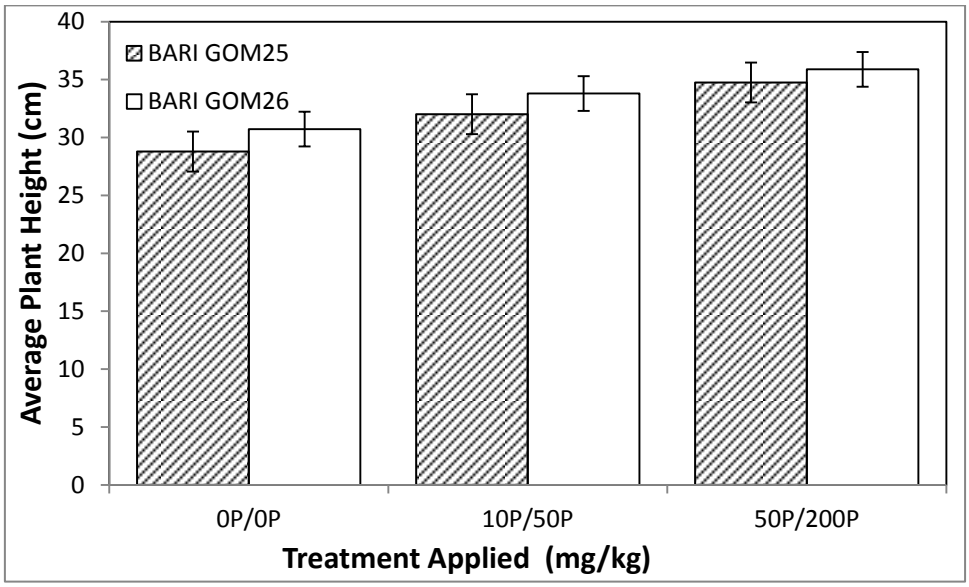

Figure 6-1: Effect of P application on average plant height of the wheat seedlings that was grown in various level of P for 28 days.

6.4.2 Shoot dry weight

Like plant height, the shoot biomass showed similar trend under different P applications. The results showed for the variety BARI GOM 25 that the maximum shoot biomass (0.90 gm/plant) was recorded in Treatment C (50P/200P mg/kg), while it was minimum (0.55 gm/plant) in Treatment A (control). Again, the results showed for the variety BARI GOM 26 that the maximum shoot biomass (0.91 gm/plant) was recorded in Treatment C (50P/200P mg/kg), while it was minimum (0.57 gm/plant) in Treatment A (control). 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 phosphorus application (Figure 6-2).

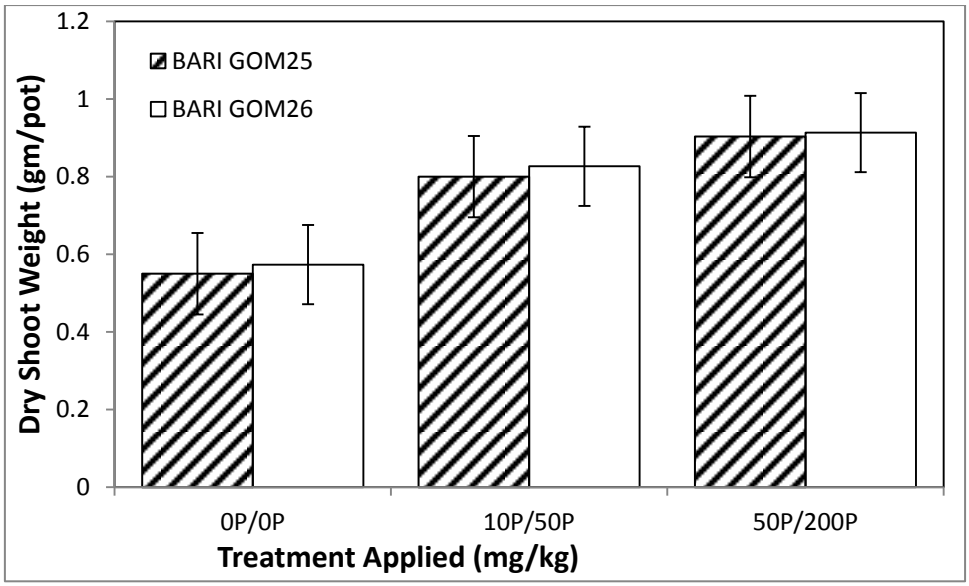

Figure 6-2: Effect of P application on dry shoot weight of the wheat seedlings that was grown in various level of P for 28 days.

6.4.3 Root dry weight

Total root biomass varied among the treatments. Total root biomass of BARI GOM 26 in Treatment C in Compartment II (200 mg/kg P) was highest (0.71gm/plant) and lowest in Treatment A-I (0.32 gm/plant), followed by gradual increase in the Treatment A-II (0.35 gm/plant), Treatment B-I (0.47 gm/plant), Treatment B-II (0.61 gm/plant) and Treatments C-I (0.62 gm/plant) (Figure 6-3). Again, for BARI GOM 25 total root biomass was highest in Treatment C in Compartment II (200 mg/kg P) (0.70gm/plant) and was lowest in Treatment A-I (0.30 gm/plant), followed by gradual increase in the Treatment A-II (0.32 gm/plant), Treatment B-I (0.45 gm/plant), Treatment B-II (0.60 gm/plant) and Treatments C-I (0.61 gm/plant). Similar to shoot dry weight, root biomass was significantly ($P \leq 0.001$) affected among all the various P application on wheat plant. But, the root biomass did not significantly ($P > 0.05$)

differ between two varieties of wheat plants. The root biomass also increased with the increasing level of phosphorus application.

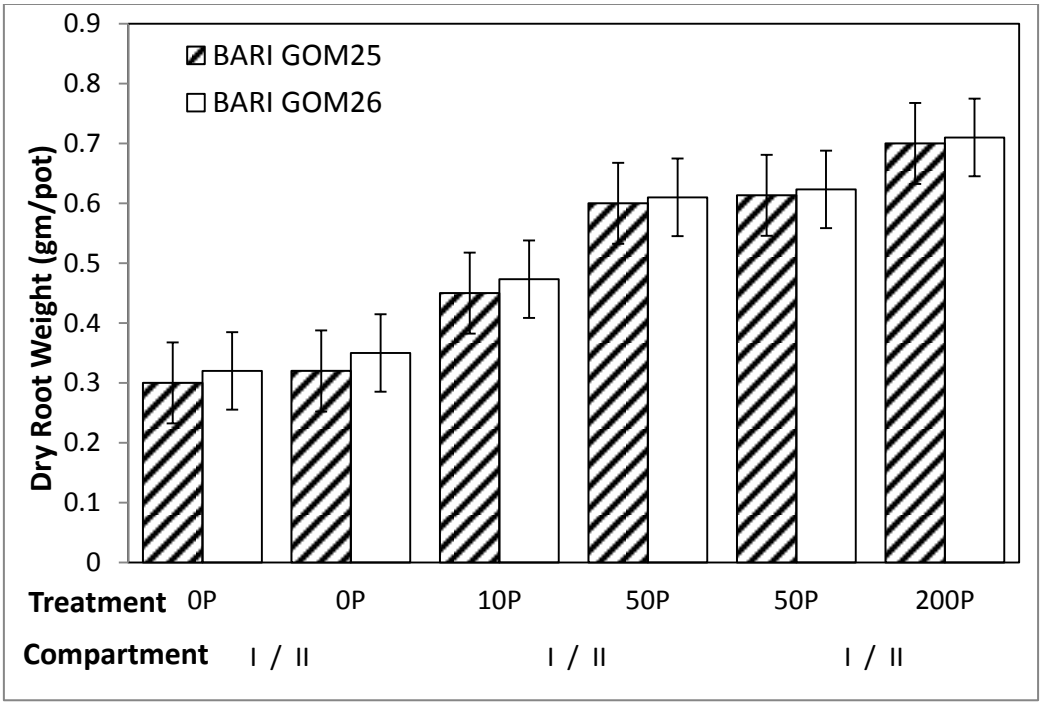

Figure 6-3: Effect of P application on dry root weight of the wheat seedlings that was grown in various level of P for 28 days.

6.4.4 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 concentrations in BARI GOM 26 was the highest and the lowest in Treatment C (4.39 gm/kg) and Treatment A (0.43 gm/kg) respectively, but intermediate in Treatments B (1.77 gm/kg) (Figure 6-4). Again, for BARI GOM 25 total soot P concentration was the highest and the lowest in Treatment C (4.23 gm/kg) and Treatment A (0.41 gm/kg) respectively, but intermediate in Treatment B (1.73 gm/kg).The shoot P

concentration of BARI GOM 25 and BARI GOM 26 were dependent on the treatments. However, the two varieties had similar responses on shoot P concentration in the different treatments.

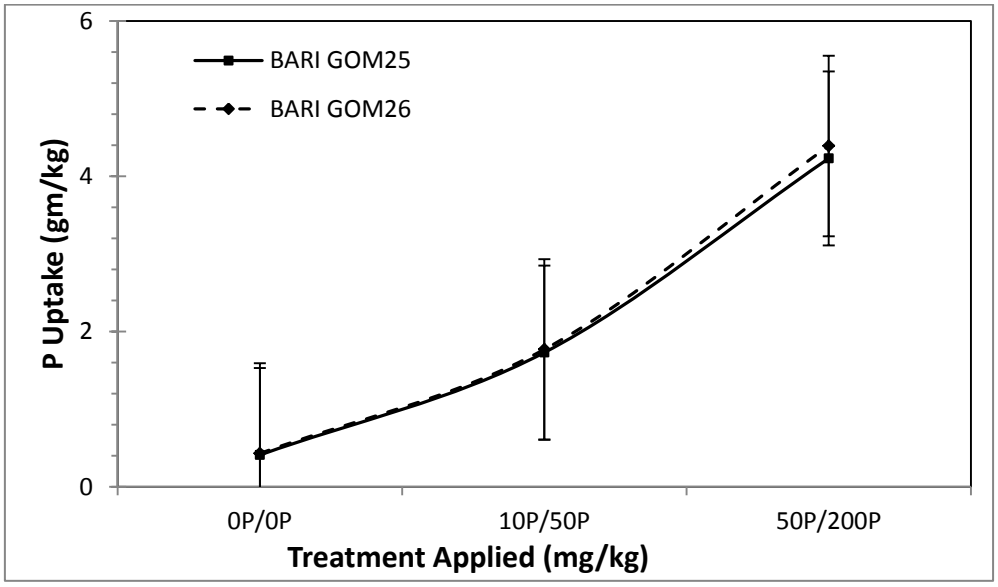

Figure 6-4: Effect of P application on P uptake of wheat shoot in various level of P for 28 days.

6.4.5 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 concentrations in BARI GOM 26 was the highest and the lowest in Treatment C in compartment II (200 mg/kg P) (3.12gm/kg) and Treatment A-I (control) (0.31 gm/plant) respectively, followed by gradual increase in Treatments C-I (1.58 gm/plant), Treatment B-II (1.44 gm/plant), Treatment B-I (0.53 gm/plant) and Treatment A-II (0.32 gm/plant) (Figure 6-5). Again, for BARI GOM 25 total root P concentrations was the highest and the lowest in Treatment C in Compartment II (200

mg/kg P) (2.97 gm/kg) and Treatment A-I (0.29 gm/plant) respectively, followed by gradual increase in Treatment C-I (1.43 gm/plant), Treatment B-II (1.39 gm/plant), Treatment B-I (0.49 gm/plant) and Treatment A-II (0.30 gm/plant). The root P concentration of BARI GOM 25 and BARI GOM 26 were dependent on the treatments. However, the two varieties had similar responses on root P concentration in the different treatments.

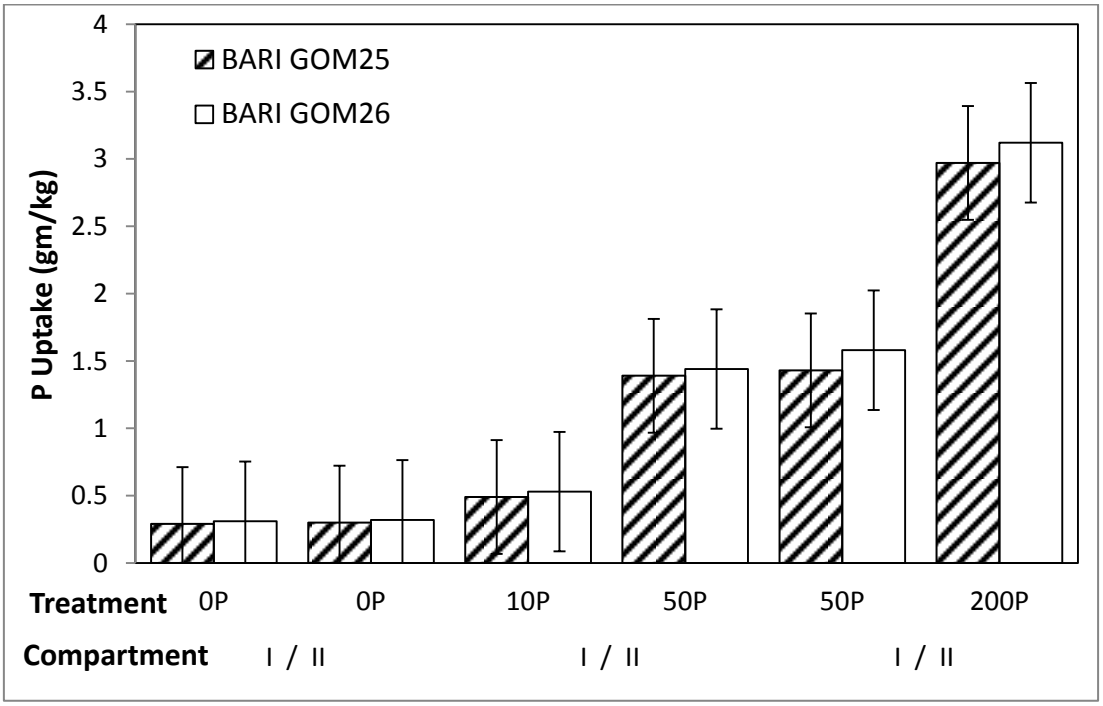

Figure 6-5: Effect of P application on P uptake of wheat root in various level of P for 28 days.

6.4.6 Total P uptake and P distribution

In both varieties of BARI GOM 25 and BARI GOM 26 similar trend in total P uptake were found. The total P uptake by wheat plant was significantly high in Treatment C from other treatments in both varieties. However, total P uptake was more than eight

times greater in Treatment C than control Treatment A. The total P uptake was greater in BARI GOM 26 than that of BARI GOM 25 in all treatments. Total P uptake in BARI GOM 26 of Treatment C and Treatment A were the highest (9.09 gm/kg) and the lowest (1.06 gm/kg) respectively, and intermediate in Treatments B (3.74 gm/kg) (Figure 6-6). Again, for BARI GOM 25 total P uptake in Treatment C and Treatment A were the highest (8.63 gm/kg) and the lowest (1.00 gm/kg) respectively, and intermediate in Treatments B (3.61 gm/kg).

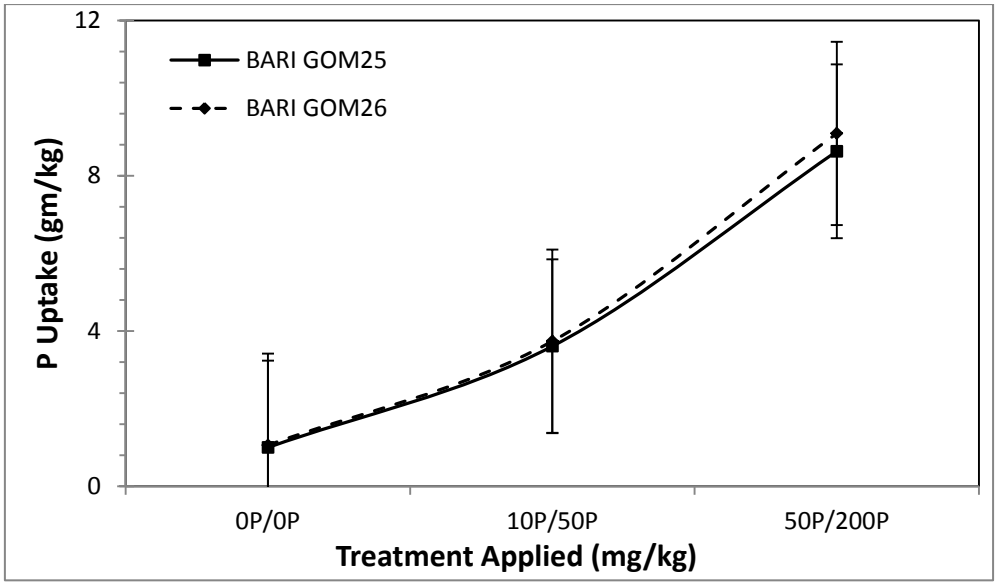

Figure 6-6: Effect of P application on P uptake of wheat plant in various level of P for 28 days.

6.5 Discussion

6.5.1 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 *et al*., 2005 and 2004). In

many soil types, P is localized in the upper soil layers and immobilized with other molecules (Chu *et al.*, 1966). It is predictably that under limiting phosphorous condition, plants that proliferate roots into these upper layers outperform varieties with deeper root systems (Zhu *et al.*, 2005 and 2004). Root proliferation and greater uptake per unit of root in the nutrient-rich zones are often considered to be compensatory responses. Again, low P availability is one of the major factors limiting crop production in acidic soils. 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 in acidic soil. 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 ($P \leq 0.001$) in this study indicates that the plant growth responses of BARI GOM 25 and BARI GOM 26 seedlings were dependent on the level of added P. In all treatments, there were no significant differences between BARI GOM 25 and BARI GOM 26 seedlings for any growth measurement. Total plant biomass in BARI GOM 26 of Treatment C increased 81% (2.25 gm/pot) in comparison with the controlled Treatment A (1.24 gm/pot). Similarly in Treatment B increased 54% (1.91 gm/pot) in comparison of Treatment A. Again, for BARI GOM 25 total plant biomass in Treatment C was increased 89% (2.22 gm/pot) in comparison with the controlled Treatment A (1.17 gm/pot). Similarly in Treatment B increased 58% (1.85 gm/pot) in comparison of Treatment A. Similar trend was found in shoot biomass and root biomass of both wheat plant varieties in this study (Table 6.4). But, internal biomass distribution in shoot and root was found different trend among all treatments (Figure 6-7). The shoot biomass was found

highest (47% of total plant biomass) in Treatment A of BARI GOM 25 and in Treatment B and Treatment C were found in deceasing order 43.2% and 40.8% of total plant biomass respectively. In case of root biomass the trend was found in increasing order in both compartments among all treatments (Table 6.4). Similarly, in BARI GOM 26 the highest percentage of shoot biomass was found in Treatment A 46.1% of total plant biomass and in Treatment B and Treatment C the percentages were found in decreasing order 43.3% and 40.7% of total plant biomass respectively. In case of root biomass the trend was found in increasing order in both compartments among all treatments (Figure 6-7). The inhibitory effect of increasing the P supply to whole root systems on the development of cluster roots of wheat plant (*Triticum aestivum* L.) is well documented (Qifu *et al.*, 2008, Pedas *et al.*, 2011, Iqbal, 2014). In our split-root study in acidic soil, 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 and root growth.

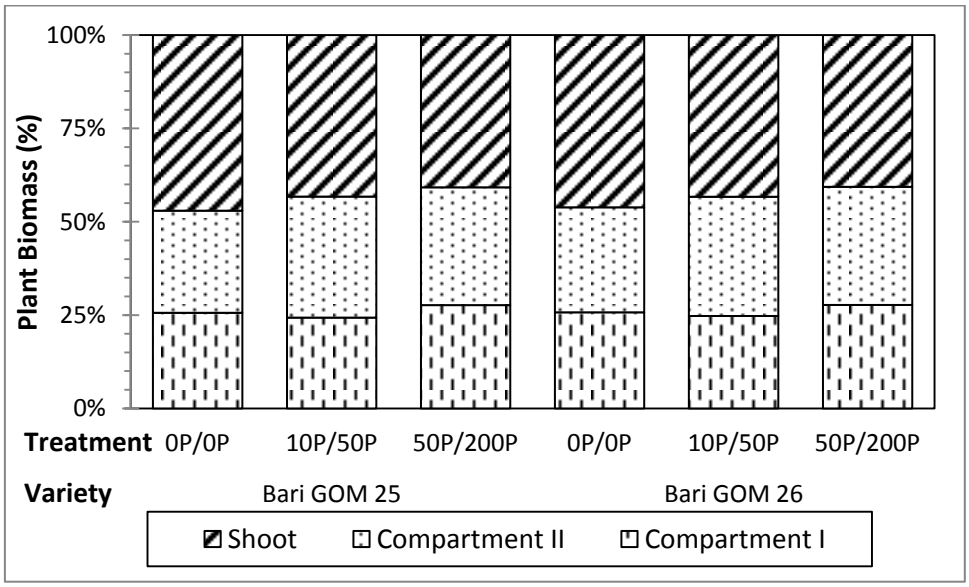

Figure 6-7: The distribution of plant biomass in different plant parts of the split-root system

Table 6.4: 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	Total Plant Biomass (gm /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 (gm/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
The 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

The root-shoot ratio is an important factor to understand growth responses of plants under elevated P applications in acidic soil. The root: shoot ratio of the wheat plant with and without treatments at the various level of P supply were analyzed (Table 6.5). Comparison of root: shoot ratio of different treatments showed an increase with the 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 Qifu *et al.*, 2011.

Table 6.5: 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

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, p< 0.05) between plant height and shoot biomass under elevated P supply indicates that plant development enhances with the application of P in soil (Figure 6-8). Similarly, a significant correlation (R^2 = 0.99, p <0.05) between plant height and root biomass under elevated P application was observed. The increase in plant growth is largely due to increase absorption of nutrients from soil solution (Son and Smith, 1988). However, the elevated P plays significant role in the growth of the wheat plant in split root system in acidic soil.

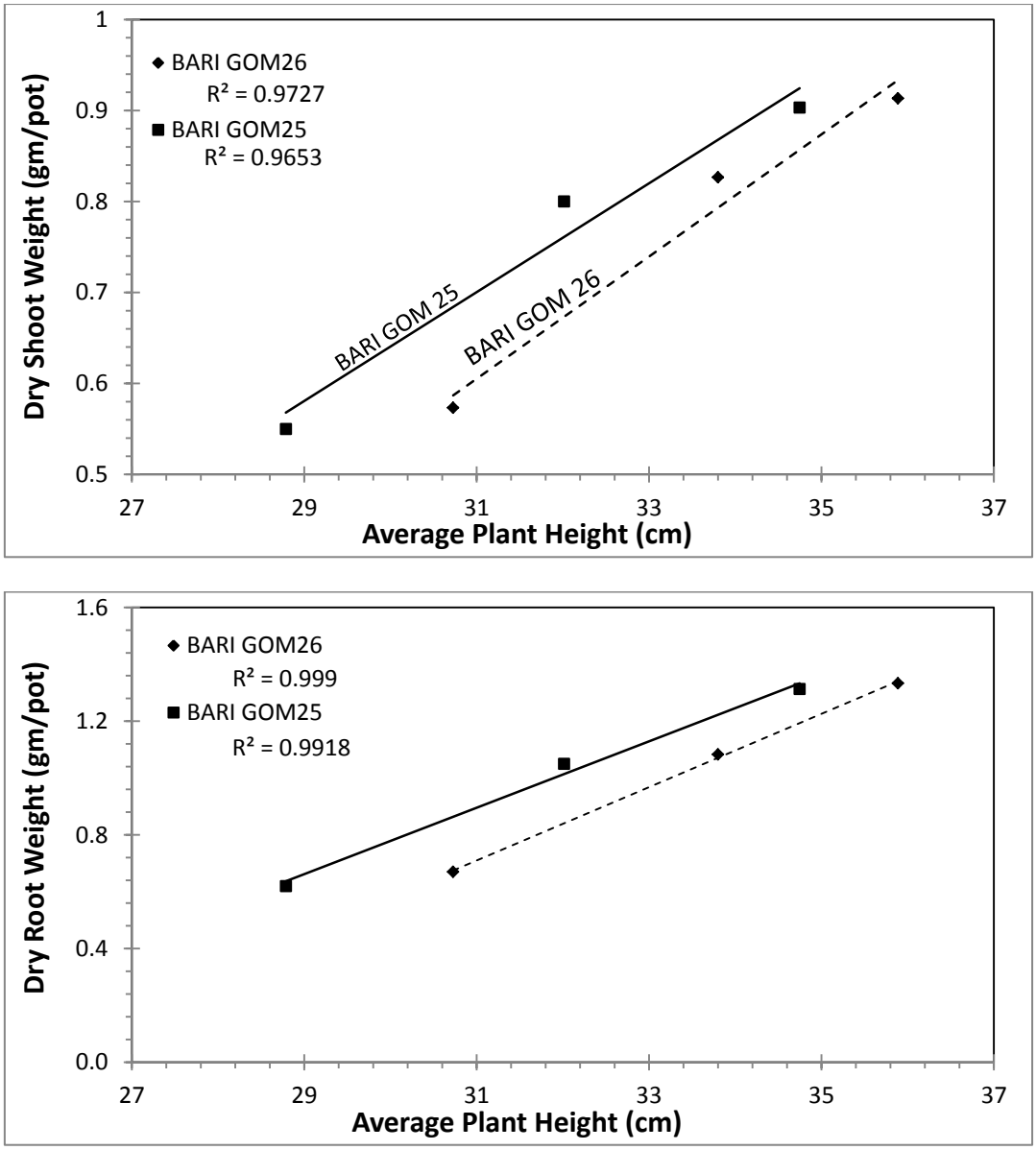

Figure 6-8: Relationship between (a) shoot dry weight and average plant height; (b) root dry weight and average plant height of wheat plant

6.5.2 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 acidic soil with a pH of 5.2 and P doses were applied directly to the soil. The shoot and root P concentration were found in increasing trend under different P application on wheat plant. Shoot and root P concentration were significantly ($P \leq 0.001$) affected among all the various P applications on wheat plant. Again, similar trends in total P uptake were found in both varieties of BARI GOM 25 and BARI GOM 26. Total plant P concentration in BARI GOM 25 of Treatment C increased more than 8 times (8.63 gm/kgt) in comparison with the controlled Treatment A (1.0 gm/kg). Similarly in Treatment B total plant P concentration increased more than three times (3.61 gm/kg) in comparison with Treatment A. Again, for BARI GOM 26, total plant biomass in Treatment C increased 9 times (9.09 gm/kg) in comparison with the controlled Treatment A (1.06 gm/kg). Similarly in Treatment B increased more than 3 times (3.74 gm/kg) in comparison of Treatment A. Similar trend was found in shoot biomass and root biomass of both wheat plant varieties in this study (Table 6.6); while internal P uptake by shoot and root was found exactly similar trend among all treatments (Figure 6-9). The highest percentages of P uptake by shoot was found in Treatment C of BARI GOM 25, 49% of total plant P uptake while in Treatment B and Treatment A it was found in deceasing order 47.9% and 41.0% respectively of total plant P uptake. Root P uptake was found in increasing order with increasing P supply in both compartments (Table 6.6). Similarly, in BARI GOM 26 the highest percentage of P uptake by shoot was found in Treatment C (48.3% of total plant P uptake) and in Treatment B and Treatment A were found in decreasing order (47.3% and 40.6% respectively of total plant P uptake). Again, root P uptake was found in increasing order with increasing P supply in both compartments (Figure 6-9). 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 of this study in split root system in acidic soil.

Table 6.6: Total P uptake in different plant parts of the split-root system and distribution of P in shoot and root of two separate compartments.

Plant parts /Variety	Total P uptake (gm /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 (gm/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
The 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

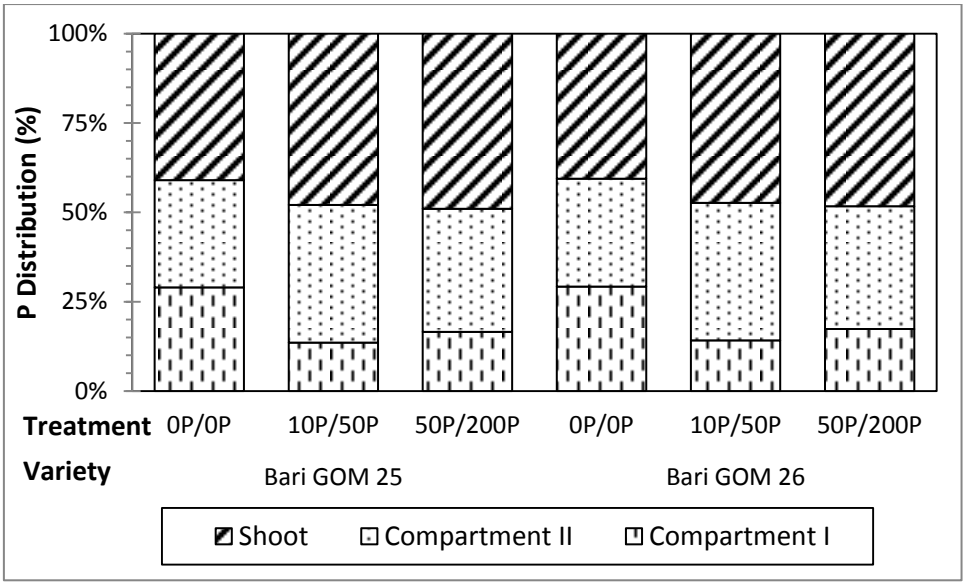

Figure 6-9: The P distribution in different plant parts of the split-root system

Several authors (Mimura *et al.*, 1996; 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 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).

Increase of the external P supply to split root 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 with, e.g. barley (Drew & Saker 1984), subterranean clover (Scott & Robson 1991), tomato (Burleigh & 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 plants, very high P supplies (200 mg P/kg KH_2PO_4) to just one crown root of wheat plants significantly increased the P concentration of compartment-I roots in respect of 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 compartment II. Studies with barley (Greenway & Gunn 1966; Clarkson & 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 in acidic soil probably provided more P for shoot unloading of P and for P assimilation in the controlled roots, resulting increased P concentrations in the roots of wheat plants. In contrast, the split-root technique is acidic soil probably provides a more stable supply of P at a lower concentration.

6.6 Conclusion

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. This study reveals that P uptake and P translocation in split root system of

the wheat plant in acidic soil. These findings indicate 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 is found in various experiments. Again, elevated P concentrations in the shoot of 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 split root system in acidic soil. Perhaps the next important leap in our 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.

Chapter 7

Understanding Phosphorus dynamics in wheat plant and growth response in a split-root system in alkaline soil

Chapter 7

7. Understanding phosphorus dynamics in wheat plant and growth response in a split-root system in alkaline soil

7.1 Introduction

Phosphorus (P) availability in most calcareous soils is very low, limiting crop production, because of the formation of sparingly soluble phosphate compounds with Ca in alkaline soils (Marschner 1995). It is estimated that more than 30% of soils cultivated globally suffer from P deficiency stress, and that the world reserves of P might be depleted by 2050 (Batjes 1997; Vance *et al.*, 2003). Phosphorus deficiency is also a critical nutritional problem in plant growth as it 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 the plants, in orthophosphate ($H_2PO_4^-$ and HPO_4^{2-}) forms (Hinsinger, 2001). Phosphorus is important in several physiological processes of plants, especially in photosynthesis, carbon metabolism, and membrane formation (Wu *et al.*, 2005). 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. This means that inorganic P is often a limiting factor in plant growth and development. 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). Phosphorus is readily translocated within the plants, moving from older to younger tissues as the plant forms cells and develops roots, stems and leaves. 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. To understand 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 in alkaline soil and to quantify how translocated phosphorus effects on wheat plant within split-root system under P efficient condition.

7.2 Materials and Methods

7.2.1 Soil and Plant

In this study, alkaline soil was used as experimental soil. The initial soil pH was 7.9 in water. The basic properties of soil are out-lined in Table 7.1. The BARI GOM 25 and BARI GOM 26 wheat varieties were used as testing plants.

Table 7.1: Properties of soils used in this experiment

Soil pH	Total N (%)	Available P (ppm)	Exchangeable K (Cmol/Kg)	Available S (ppm)	Available Zn (ppm)	Organic matter (%)
7.9	0.03	14.3	0.21	5.6	11.55	0.55

7.2.2 Experimental design

The split-root experiment was conducted with the treatments described in Table 7.2. The BARI GOM 25 and BARI GOM 26 were compared. The treatments were replicated three times. The KH_2PO_4 chemical was used as a P source. 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 30°C for 7 days then KH_2PO_4 as per P doses were applied directly to the soil in each cup and mixed thoroughly before sowing. The total experiment was conducted in Research Laboratories, Department of Agronomy & Agricultural Extension, Rajshahi University, Rajshahi.

Table 7.2: Split-root system with different treatments

Treatment	Symbols	Treatment symbols		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

7.2.3 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.

7.2.4 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 was continued for 28 days.

7.2.5 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-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. A 20 ml water was added to each compartment every day and watering was stopped 3 days before harvesting.

7.2.6 Harvesting

The experimental plants were harvested 28 days after transplanting. The shoots were cut 0.5 cm above the base part of the stem uniformly. Then the roots were cut 0.5 cm below the base part and separated carefully into two halves as previously marked. Soils from two root halves removed carefully so that the roots could not be toned 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 the roots. The washed roots were oven dried at 70°C for 3 days. Shoots were also over dried at the same temperature for the same time. After drying, the root and shoot samples were weighed and stored for analysis.

7.3 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 percloric acids (4: 1), the concentration of P in root and shoot materials were determined using the vanadomolybdate method after digestion in a mixture of concentrated nitric and perchloric acids (4:1). Colorimetric method for the determination of phosphorous 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 DI water. The absorbance reading was used at 470 nm (Iqbal *et al.*, 2010).

7.3.1 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 H_2O_2 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 Asija, 1956). The distillate was collected in 20 ml of 2% boric acid solution with methylred and bromocresol green indicator and titrated with 0.02 N

sulphuric acid (H_2SO_4) (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 NH_4OAC and determined by an atomic absorption spectrometer (Biswas *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 $NaHCO_3$ 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).

7.3.2 Statistical analysis

Shoot parameters were analyzed by two-way ANOVA (Treatment × Variety) and root parameters were analyzed 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 (Lawes Agricultural Trust, UK).

7.4 Results

All the plant growth and P-uptake parameters were highly significant ($P \leq 0.001$) under P levels (Table 7.3). Similarly, significant differences among varieties were observed in relation to all the growth and P-uptake parameters.

Table 7.3: Significance levels for the main and interactive effect of P and wheat 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)	***	n.s.	***	**	**
Compartment (C)	-	-	-	***	***
T×V	***	n.s.	***	n.s.	n.s.
T×C	-	-	-	***	***
C×V	-	-	-	n.s.	n.s.
T×V×C	-	-	-	n.s.	n.s.

Where n.s., ** and *** represent probability of > 0.05, ≤ 0.01 and ≤ 0.001, respectively, and '-' (dash) indicates no data available.

7.4.1 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 7-1. The results showed for the variety BARI GOM 25 that the maximum plant height (30.76 mm) was recorded in treatment C (50P/200P mg/kg), while it was minimum (23.87 mm) in Treatment A (control). Again, the results showed for the variety BARI GOM 26 that the maximum plant height (30.99 mm) was recorded in Treatment C (50P/200P mg/kg), while it was minimum (25.13 mm) in Treatment A (control). Plant height was significantly ($P \leq 0.001$) affected among all the various P applications and variety of wheat plants. It also increased with the increasing level of phosphorus application. Hence, among low level of various phosphorous application, phosphate had the gradual increasing effect on plant height with increasing P applications, while at high level P resulted in maximum plant height.

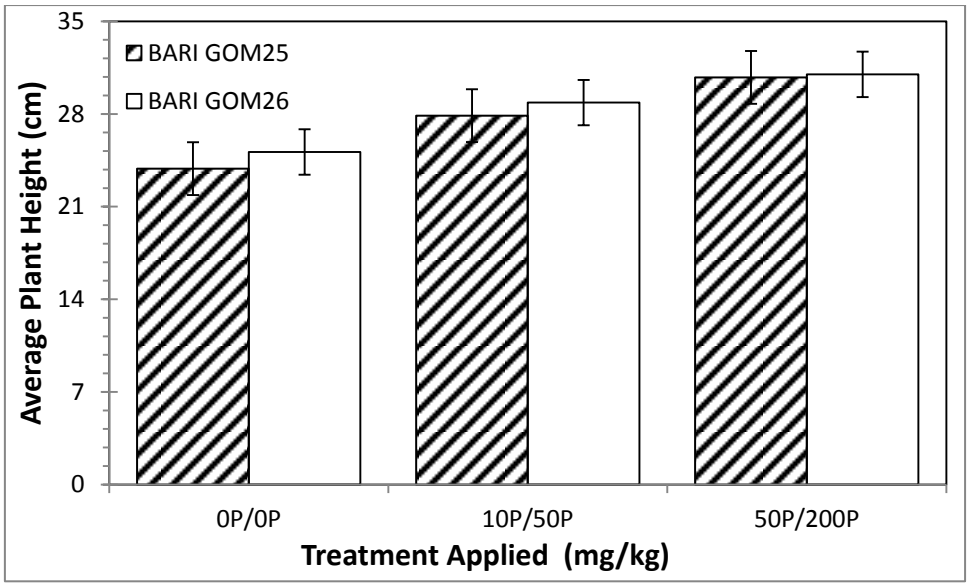

Figure 7-1: Effect of P application on average plant height of the wheat seedlings that was grown in various level of P for 28 days.

7.4.2 Shoot dry weight

Like plant height, the shoot biomass showed similar trend under different P applications. The results showed for the variety BARI GOM 25 that the maximum shoot biomass (0.60 gm/pot) was recorded in Treatment C (50P/200P mg/kg), while it was minimum (0.40 gm/pot) in Treatment A (control). Again, the results showed for the variety BARI GOM 26 that the maximum shoot biomass (0.62 gm/pot) was recorded in Treatment C (50P/200P mg/kg), while it was minimum (0.42 gm/pot) in Treatment A (control). 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 plants. The shoot biomass also increased with the increased level of phosphorus application (Figure 7-2).

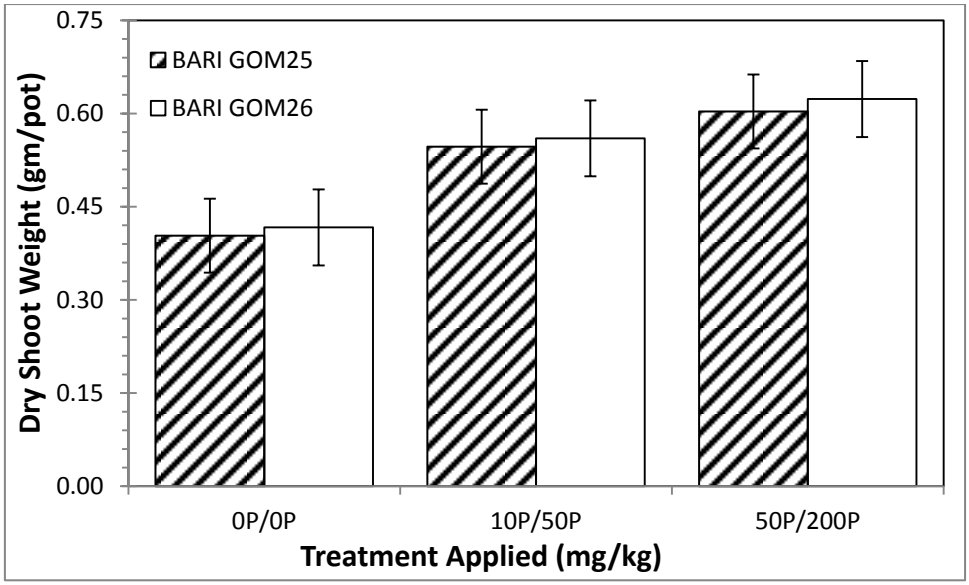

Figure 7-2: Effect of P application on dry shoot weight of the wheat seedlings that was grown in various level of P for 28 days.

7.4.3 Root dry weight

Total root biomass varied among the treatments. Total root biomass of BARI GOM 26 in Treatment C on Compartment II (200 mg/kg P) was highest (0.57 gm/pot) and lowest in Treatment A-II (0 mg/kg P) (0.23 gm/pot), followed by gradual increase in the Treatment A-I (0 mg/kg P) (0.26 gm/pot),Treatment B-I (10 mg/kg P) (0.32 gm/pot), Treatment B-II (50 mg/kg P) (0.46 gm/pot) and Treatments C-I (50 mg/kg P) (0.47 gm/pot) (Figure 7-3). Again, for BARI GOM 25 total root biomass was highest in Treatment C on Compartment II (200 mg/kg P) (0.55 gm/pot) and lowest in Treatment A-II (0 mg/kg P) (0.22 gm/pot), followed by gradual increase in the Treatment A-I (0 mg/kg P) (0.25 gm/pot), Treatment B-I (10 mg/kg P) (0.30 gm/pot), Treatment B-II (50 mg/kg P) (0.45 gm/pot) and Treatments C-I (50 mg/kg P) (0.46 gm/pot). Similar to shoot dry weight, root biomass was significantly ($P \leq 0.001$) affected among all the various P application on wheat plant. But, the root biomass

affected moderately ($P \leq 0.01$) between two varieties of wheat plants. The root biomass also increased with the increasing level of phosphorus application.

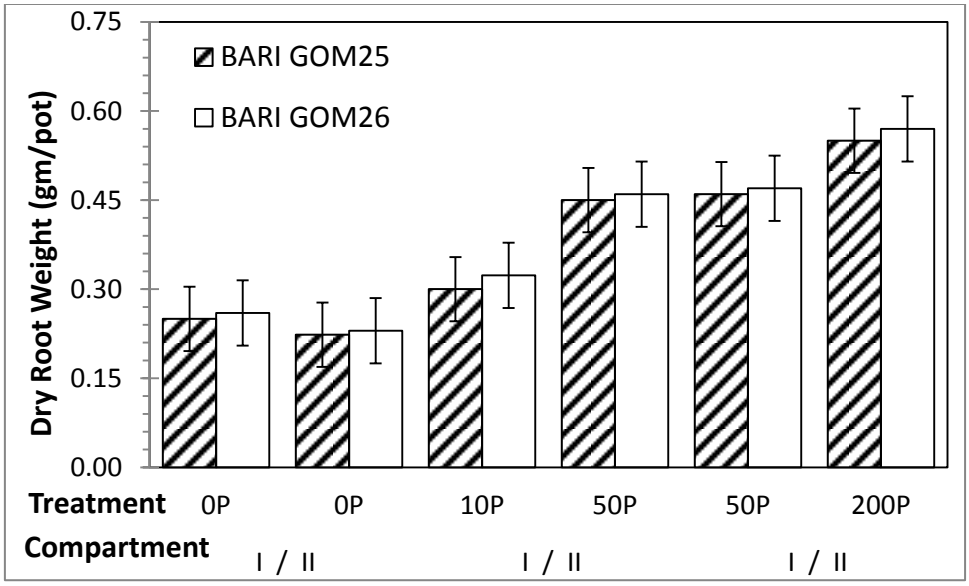

Figure 7-3: Effect of P application on dry root weight of the wheat seedlings that was grown in various level of P for 28 days.

7.4.4 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 concentrations in BARI GOM 26 was the highest and the lowest in Treatment C (50P/200P mg/kg) (4.27 gm/kg) and Treatment A (0P/0P mg/kg) (0.40 gm/kg) respectively, but intermediate in Treatments B (10P/50P mg/kg) (1.69 gm/kg) (Figure 7-4). Again, for BARI GOM 25 total soot P concentrations was the highest and the lowest in Treatment C (50P/200P mg/kg) (4.02 gm/kg) and Treatment A (0P/0P mg/kg) (0.39 gm/kg) respectively, but intermediate in Treatments B (10P/50P mg/kg)

(1.67 gm/kg). The shoot P concentration of BARI GOM 25 and BARI GOM 26 were dependent on the treatments. However, the two varieties had similar responses on shoot P concentration in the different treatments.

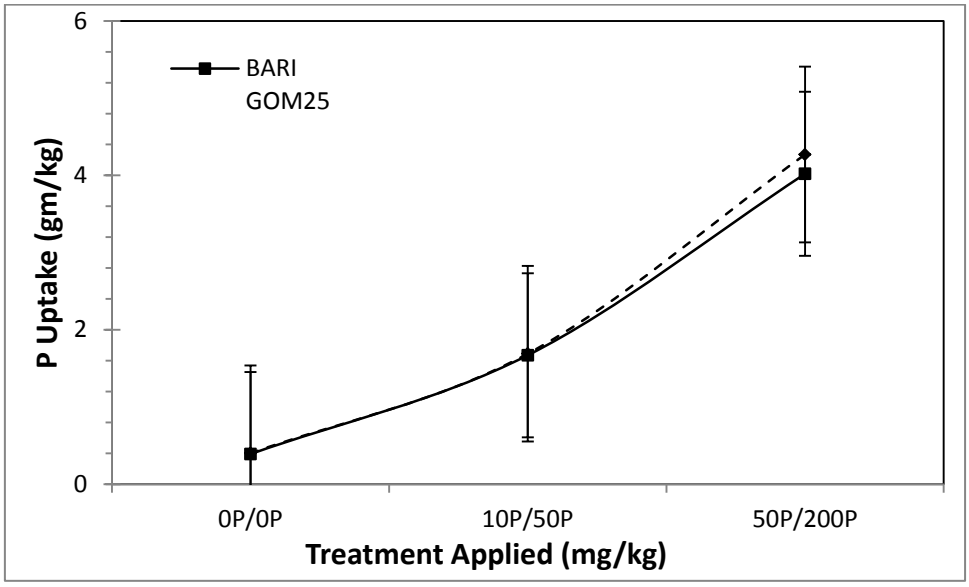

Figure 7-4: Effect of P application on P uptake of wheat shoot in various level of P for 28 days.

7.4.5 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 concentrations in BARI GOM 26 was the highest and the lowest in Treatment C in Compartment II (200 mg/kg P) (2.97 gm/kg) and Treatment A-II (0 mg/kg P) (0.28 gm/plant) respectively, followed by gradual increase in Treatment A-I (0 mg/kg P) (0.29 gm/plant) , Treatment B-I (10 mg/kg P) (0.47 gm/plant), Treatment B-II (50 mg/kg P) (1.38 gm/plant) and Treatments C-I (50 mg/kg P) (1.53 gm/plant) (Figure 7-5). Again, for BARI GOM 25 total root P concentrations was the highest and the

lowest in Treatment C in Compartment II (200 mg/kg P) (2.89 gm/kg) and Treatment A-I (0 mg/kg P) (0.26 gm/plant) respectively, followed by gradual increase in Treatment A-II (0 mg/kg P) (0.27 gm/plant) , Treatment B-I (10 mg/kg P) (0.43 gm/plant), Treatment B-II (50 mg/kg P) (1.31 gm/plant) and Treatment C-I (50 mg/kg P) (1.35 gm/plant). The root P concentration of BARI GOM 25 and BARI GOM 26 were dependent on the treatments. However, the two varieties had similar responses on root P concentration in the different treatments.

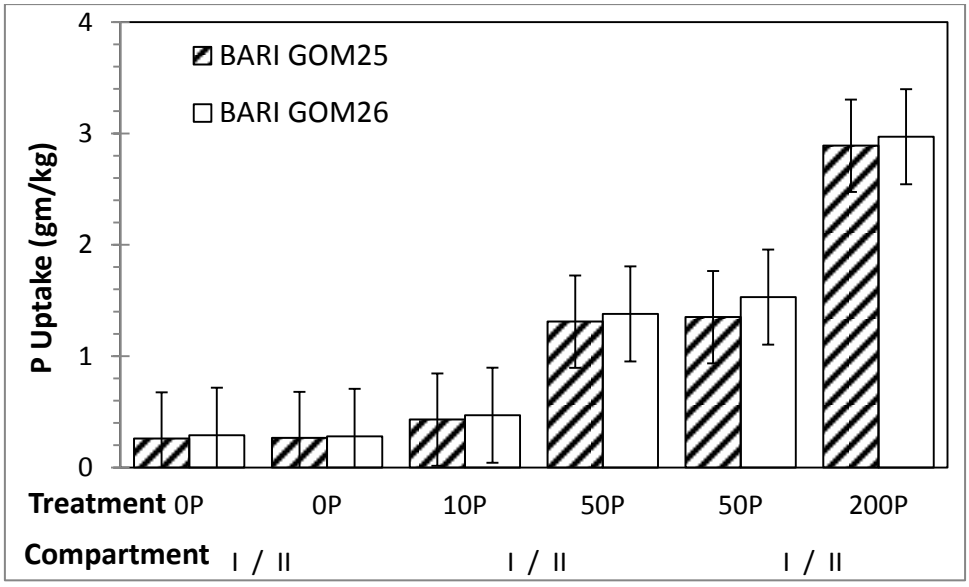

Figure 7-5: Effect of P application on P uptake of wheat root in various level of P for 28 days.

7.4.6 Total P uptake and P distribution

In both varieties of BARI GOM 25 and BARI GOM 26 similar trend in total P uptake were found. The total P uptake by plant was significantly high in Treatment C (50P/200P mg/kg) from other treatments in both varieties. However, total P uptake was more than eight times greater in Treatment C (50P/200P mg/kg) than control

Treatment A (0P/0P mg/kg). The total P uptake was greater in BARI GOM 26 than BARI GOM 25 in all treatments. Total P uptake in BARI GOM 26 of Treatment C (50P/200P mg/kg) and Treatment A (0P/0P mg/kg) were the highest (8.77 gm/kg) and the lowest (0.97 gm/kg), and intermediate in Treatments B (10P/50P mg/kg) (3.54 gm/kg) (Figure 7-6). Again, for BARI GOM 25 total P uptake in Treatment C (50P/200P mg/kg) and Treatment A (0P/0P mg/kg) were the highest (8.26gm/kg) and the lowest (0.92 gm/kg), and intermediate in Treatments B (10P/50P mg/kg) (3.41 gm/kg).

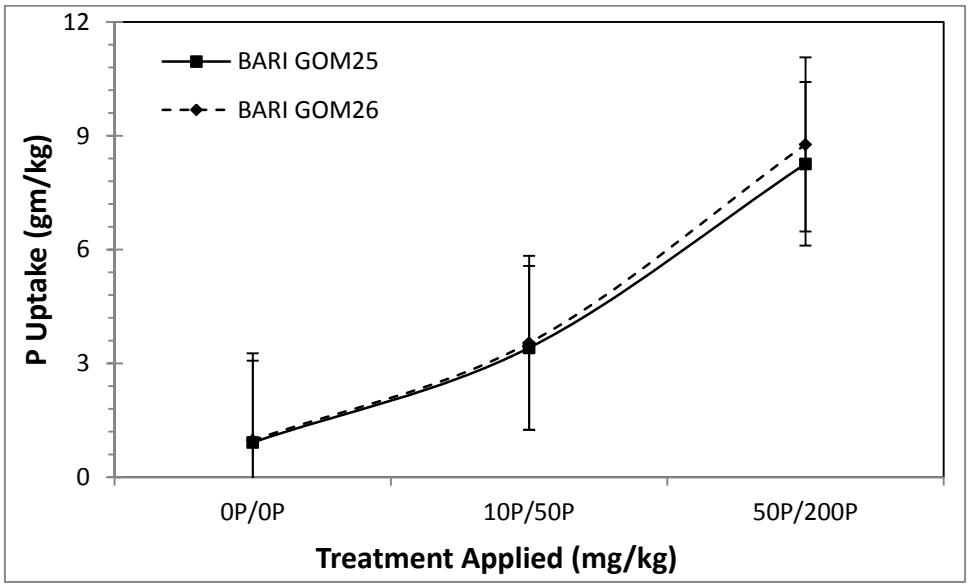

Figure 7-6: Effect of P application on P uptake of wheat plant in various level of P for 28 days.

7.5 Discussion

7.5.1 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 *et al.*, 2005 and 2004). In

many soil types, P is localized to the upper soil layers and immobilized with other molecules (Chu *et al.*, 1966). It is predictably that under limiting phosphorous condition, plants that proliferate roots into these upper layers outperform varieties with deeper root systems (Zhu *et al.*, 2005 and 2004). Root proliferation and greater uptake per unit of root in the nutrient-rich zones are often considered to be compensatory responses. According to Raghothama *et al.*, (2005) and Rahim *et al.*, (2007), P deficiency is very common in alkaline calcareous soils. 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 in alkaline soil. 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 ($P \leq 0.001$) in this study indicates that the plant growth responses of BARI GOM 25 and BARI GOM 26 seedlings were dependent on the level of added P. In all treatments, there were no significant differences between BARI GOM 25 and BARI GOM 26 seedlings for any growth measurement. Total plant biomass in BARI GOM 26 of Treatment C increased 82% (1.66 gm/pot) in comparison with the controlled Treatment A (0.91 gm/pot). Similarly in Treatment B increased 47% (1.34 gm/pot) in comparison of Treatment A. Again, for BARI GOM 25 total plant biomass in Treatment C was increased 83% (1.61 gm/pot) in comparison with the controlled Treatment A (0.88 gm/pot). Similarly in Treatment B increased 48% (1.30 gm/pot) in comparison of Treatment A. Similar trend was found in shoot biomass and root biomass of both wheat plant varieties in this study (Table 7.4). But, internal biomass distribution in shoot and root were found different trend among all treatments (Figure

7-7). The shoot biomass was found highest (46% of total plant biomass) in Treatment A of BARI GOM 25, and in Treatment B and Treatment C the percentages were found in deceasing order 42.2% and 37.4% respectively of total plant biomass. In case of root biomass the trend was found in increasing order in both compartments among all treatments (Table 7.4). Similarly, in BARI GOM 26 the highest percentage of shoot biomass was found in Treatment A 46.0% of total plant biomass and in Treatment B and Treatment C the percentages were found in decreasing order 41.7% and 37.5% of total plant biomass respectively. In case of root biomass the trend was found in increasing order in both compartments among all treatments (Figure 7-7). The inhibitory effect of increasing the P supply to whole root systems on the development of cluster roots of wheat plant (*Triticum aestivum* L.) is well documented (Qifu *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 and root growth.

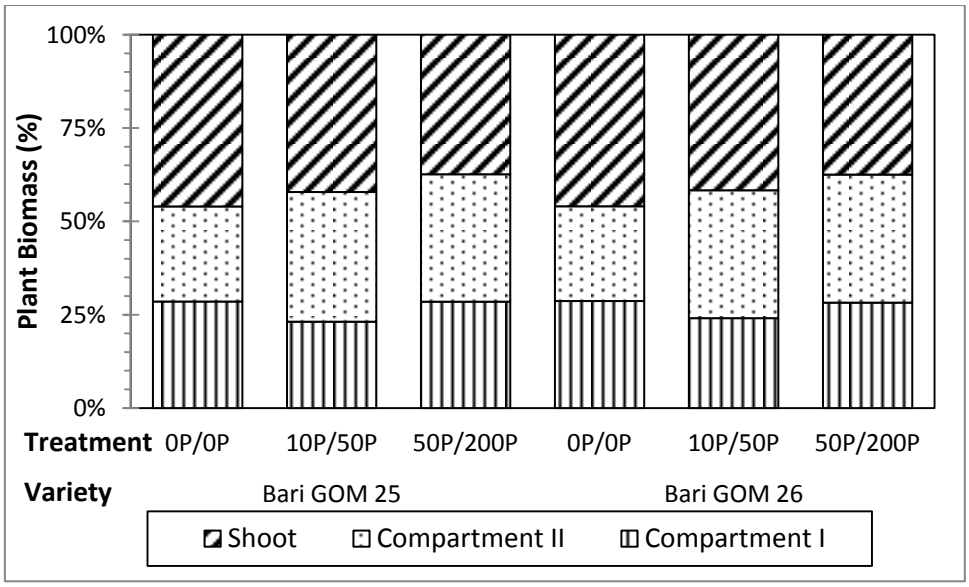

Figure 7-7: The distribution of plant biomass in different plant parts of the split-root system

Table 7.4: 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 /Varieties	Total Plant Biomass (gm /pot)		
	Treatment A	Treatment B	Treatment C
BARI GOM 25	0.88	1.30	1.61
BARI GOM 26	0.91	1.34	1.66
Total Biomass (gm/pot) in different plant parts of the split-root system			
BARI GOM 25			
Shoot	0.40	0.55	0.60
Compartment-I	0.25	0.30	0.46
Compartment-II	0.22	0.45	0.55
BARI GOM 26			
Shoot	0.42	0.56	0.62
Compartment-I	0.26	0.32	0.47
Compartment-II	0.23	0.46	0.57
The distribution of Biomass (%) in shoot and roots grown in two separate soil compartments (I and II)			
BARI GOM 25			
Shoot	46.0	42.2	37.4
Compartment-I	28.5	23.1	28.5
Compartment II	25.5	34.7	34.1
BARI GOM 26			
Shoot	46.0	41.7	37.5
Compartment I	28.7	24.1	28.3
Compartment II	25.4	34.2	34.3

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 7.5). Comparison of root: shoot ratio of different treatments showed an increase with the 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 Qifu *et al.*, 2011.

Table 7.5: Root biomass, shoot biomass and root/shoot ratio of two wheat varieties across different P applications

Varieties	Treatment	P rate (mg/kg)	Biomass Production (mg/plant)		Root-shoot ratio
			Shoot	Root	
BARI GOM 25	T1	0P/0P	0.40	0.47	1.17
	T2	10P/50P	0.55	0.75	1.37
	T3	50P/200P	0.60	1.01	1.67
BARI GOM 26	T1	0P/0P	0.42	0.49	1.18
	T2	10P/50P	0.56	0.78	1.40
	T3	50P/200P	0.62	1.04	1.67

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, p< 0.05) between plant height and shoot biomass under elevated P supply indicates that plant development enhances with the application of P in soil (Figure 7-8). Similarly, a significant correlation (R^2 = 0.99, p<0.05) between plant height and root biomass under elevated P application was observed. The increase in plant growth is largely due to increase absorption of nutrients from soil solution (Son and Smith, 1988). However, the elevated P plays significant role in the growth of the wheat plant.

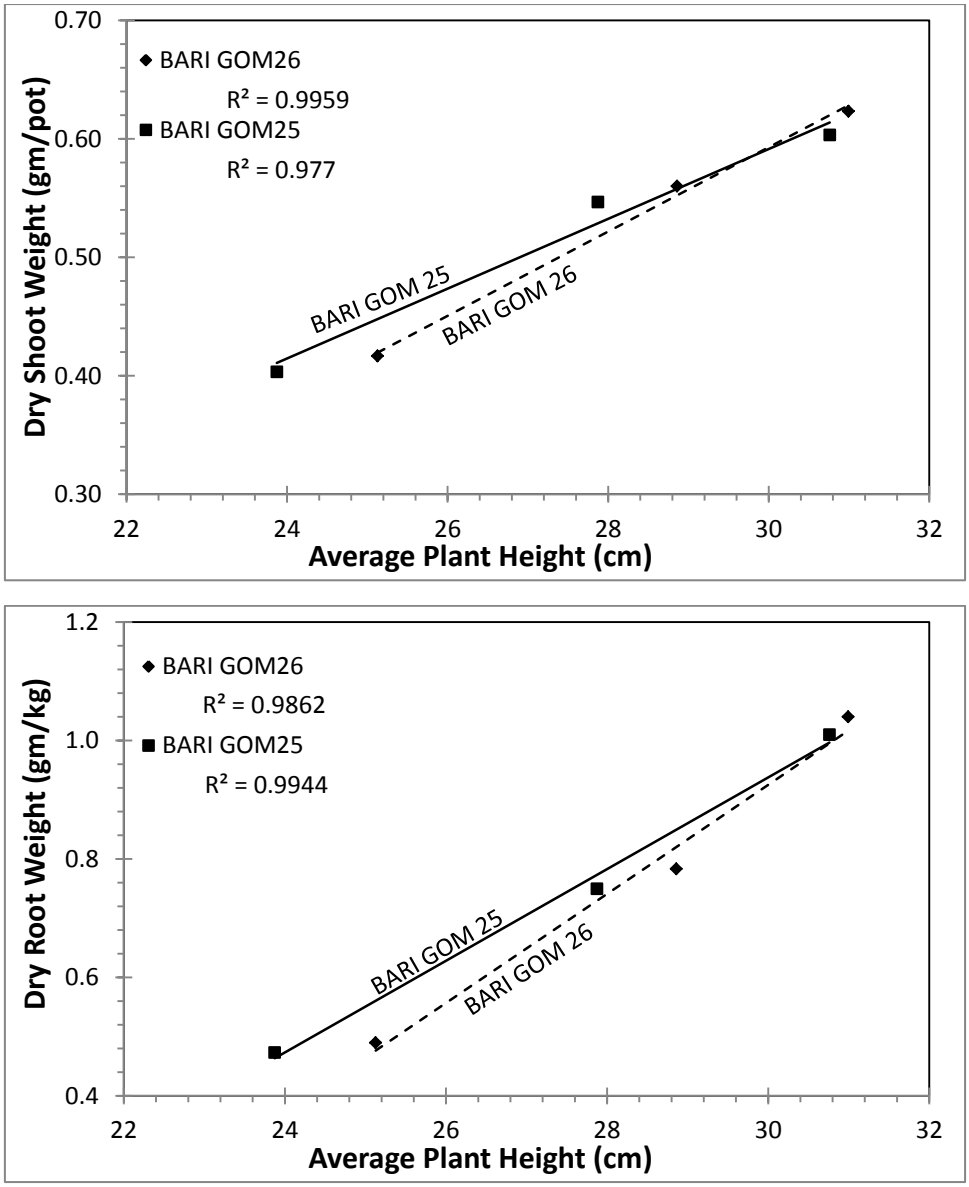

Figure 7-8: Relationship between (a) shoot dry weight and average plant height; (b) root dry weight and average plant height of wheat plant

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

In general, plants grow better when partially soluble phosphate is applied in comparison to the soluble P source. The study was conducted in alkaline soil and P doses were applied directly to the soil. Hence, the shoot and root P concentration were

found in increasing trend under different P application on wheat plant. Shoot and root P concentration were significantly ($P \leq 0.001$) affected among all the various P applications on wheat plants. Again, similar trends in total P uptake were found in both varieties of BARI GOM 25 and BARI GOM 26. Total plant P concentration in BARI GOM 25 of Treatment C increased more than 8 times (8.26 gm/kg) in comparison with the controlled Treatment A (0.92 gm/kg). Similarly in Treatment B, total plant P concentration increased more than three times (3.41 gm/kg) in comparison with Treatment A. Again, for BARI GOM 26, total plant biomass in Treatment C increased 9 times (8.77 gm/kg) in comparison with the controlled Treatment A (0.97 gm/kg). Similarly in Treatment B, the total biomass increased more than 3 times (3.54 gm/kg) in comparison with Treatment A. Similar trend was found in shoot P concentration and root P concentration of both wheat plant varieties in this study (Table 7.6); while internal P uptake by shoot and root was found exactly similar trend among all treatments (Figure 7-9). The highest percentages of P uptake by shoot was found in Treatment B of BARI GOM 25, 49.0% of total plant P uptake while in Treatment C and Treatment A it was found in deceasing order 48.7% and 42.6% respectively of total plant P uptake. Root P uptake was found in increasing order with increasing P supply in both compartments (Table 7.6). Similarly, in BARI GOM 26 the highest percentage of P uptake by shoot was found in Treatment C (48.7% of total plant P uptake) and in Treatment B and Treatment A were found in decreasing order (47.7% and 41.2% respectively of total plant P uptake). Again, root P uptake was found in increasing order with increasing P supply in both compartments (Figure 7-9). This percentage distribution differences in the total root and shoot P uptake among the three P treatments are due to elevated P supply which directly interferes with shoot root P status.

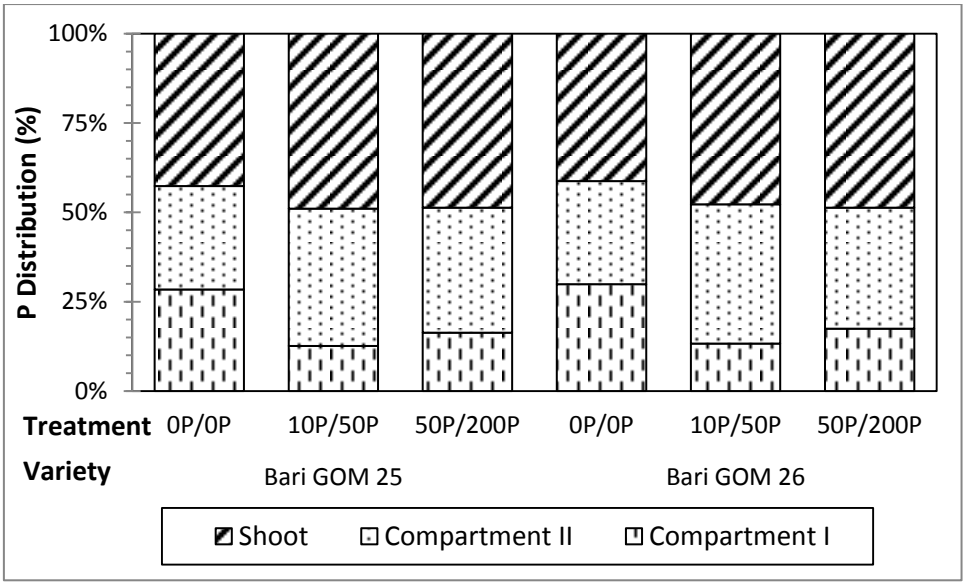

Figure 7-9: The P distribution in different plant parts of the split-root system

Table 7.6: Total P uptake in different plant parts of the split-root system and distribution of P in shoot and root of two separate compartments.

Plant parts /Varieties	Total P uptake (gm /kg)		
	Treatment A	Treatment B	Treatment C
BARI GOM 25	0.92	3.41	8.26
BARI GOM 26	0.97	3.54	8.77
Total P uptake (gm/kg) in different plant parts of the split-root system			
BARI GOM 25			
Shoot	0.39	1.67	4.02
Compartment-I	0.26	0.43	1.35
Compartment-II	0.27	1.31	2.89
BARI GOM 26			
Shoot	0.40	1.69	4.27
Compartment-I	0.29	0.47	1.53
Compartment-II	0.28	1.38	2.97
The distribution of P (%) in shoot and roots grown in two separate soil compartments (I and II)			
BARI GOM 25			
Shoot	42.6	49.0	48.7
Compartment-I	28.4	12.6	16.3
Compartment II	29.0	38.4	35.0
BARI GOM 26			
Shoot	41.2	47.7	48.7
Compartment I	29.9	13.3	17.4
Compartment II	28.9	39.0	33.9

Mimura *et al.*, 1996; 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 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).

Increase of the external P supply to split root from 0 mg P/kg to 200 mg P/kg significantly increased the P concentration in those roots, and in 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 & Saker 1984), subterranean clover (Scott & Robson 1991), tomato (Burleigh & 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 plants, very high P supplies (200 mg P/kg KH_2PO_4) to just one crown root of wheat

plants significantly increased the P concentration of compartment-I roots in respect of Treatment B compartment II. It was expected that in Treatment C, plants would be able to translocates P from the roots in compartment I to those compartment II. Studies with barley (Greenway & Gunn 1966; Clarkson & 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 in alkaline soil probably provides a more stable supply of P at a lower concentration.

7.6 Conclusion

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. This study reveals that P uptake and P translocation in split root system of the wheat plant in alkaline soil. These findings indicate 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 is found in various experiments. Again, elevated P concentrations in the shoot of 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 split root system in alkaline soil. Perhaps the next important leap in our 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.

Chapter 8

Understanding Phosphorus translocation in wheat plant and growth response in a split-root system

Chapter 8

8. Understanding phosphorus translocation in wheat plant and growth response in a split-root system

8.1 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 ($H_2PO_4^-$ and HPO_4^{2-}) forms (Hinsinger, 2001). Phosphorus is important in several physiological processes of plants, especially in photosynthesis, carbon metabolism, and membrane formation (Wu *et al.*, 2005). 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. 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 symbiosis with arbuscular mycorrhizas or ectomycorrhizas (Péret *et al.*, 2011; Smith *et al.*, 2011). Phosphorus is readily translocated within the plants, moving from older to younger tissues as the plant forms cells and develops roots, stems and leaves. 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. 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 and to quantify how translocated phosphorus effects on wheat plant within split-root system under P efficient condition.

8.2 Materials and Methods

8.2.1 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 acidic region of Bangladesh and the soil type II (alkaline) having pH 7.9 was collected from alkaline soil region of Bangladesh. The basic properties of soil are out-lined in Table 8.1. BARI GOM 25 and BARI GOM 26 wheat (*Triticum aestivum* L.) varieties were used as testing plants.

Table 8.1: Properties of soils used in this experiment

Soil Type	Soil pH	Total N (%)	Available P (ppm)	Exchangeable K (Cmol/Kg)	Available S (ppm)	Available Zn (ppm)	Organic matter (%)
Soil I (Acidic)	5.2	0.05	10.2	0.2	19.5	0.59	0.85
Soil II (Alkaline)	7.9	0.03	14.3	0.21	5.6	11.55	0.55

8.2.2 Experimental design

The split-root experiment was conducted with the treatments described in Table 8.2. The BARI GOM 25 and BARI GOM 26 were compared. The treatments were replicated three times. The KH_2PO_4 chemical was used as a P source. 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 30°C for 7 days, 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 & Agricultural Extension, Rajshahi University, Rajshahi.

Table 8.2: Split-root system with different treatments

Treatment	Symbols	Treatment symbols		P level	
		Compartment 1	Compartment 2	Compartment 1	Compartment 2
		Alkaline Soil	Acidic Soil	Alkaline soil	Acidic Soil
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
D	100P/400P	100P	400P	100 mg P/kg	400 mg P/kg

8.2.3 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.

8.2.4 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.

8.2.5 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-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. A 20 ml water was added to each compartment every day and watering was stopped 3 days before harvesting.

8.2.6 Harvesting

The experimental plants were harvested 28 days after transplanting. The shoots were cut into 0.5 cm above the base part of the stem uniformly. Then the roots were cut 0.5 cm below the base part and separated carefully into two halves as previously marked. Soils from two root halves 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 over dried at the same temperature for the same time. After drying, the root and shoot samples were weighed and stored for analysis.

8.3 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 concentration of P in root and shoot materials were determined using the vanadomolybdate method. Colorimetric method for the determination of phosphorous 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 DI water. The absorbance reading was used at 470 nm (Iqbal *et al.*, 2010).

8.3.1 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 H_2O_2 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 Asija, 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 (H_2SO_4) (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 NH_4OAC and determined by an atomic absorption spectrometer (Biswas *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 $NaHCO_3$ 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).

8.3.2 Statistical analysis

Shoot and root parameters were analyzed 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 (Lawes Agricultural Trust, UK).

8.4 Results

All the plant growth and P-uptake parameters were highly significant ($P \leq 0.001$) under P levels (Table 8.3). Similarly, significant differences among varieties were observed in relation with all the growth and P-uptake parameters.

Table 8.3: Significance levels for the main and interactive effect of P and wheat 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)	***	n.s.	***	n.s.	n.s.
Compartment (C)	-	-	-	n.s.	*
T×V	***	n.s.	***	n.s.	n.s.
T×C	-	-	-	n.s.	n.s.
C×V	-	-	-	n.s.	n.s.
T×V×C	-	-	-	*	n.s.

Where n.s., ** and *** represent probability of > 0.05, ≤ 0.01 and ≤ 0.001, respectively, '-' indicates no data available.

8.4.1 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 8-1. The results showed for the variety BARI GOM 25 that the maximum plant height (30.83 mm) was recorded in Treatment C (50P/200P mg/kg), while minimum (14.78 mm) was found in Treatment D (100P/400P mg/kg). Again, the results showed for the variety BARI GOM 26 that the maximum plant height (30.85 mm) was recorded in Treatment C (50P/200P mg/kg), while minimum (16.77 mm) was found in Treatment D (100P/400P mg/kg). Plant height was significantly ($P \leq 0.001$) affected among all the various P applications and variety of wheat plants. It also increased with the increasing level of phosphorus application, but at high level P resulted in minimum plant height. Hence, among low level of various phosphorous application, phosphate had the gradual increasing effect on plant height with increasing P applications, but at high level P resulted in minimum plant height.

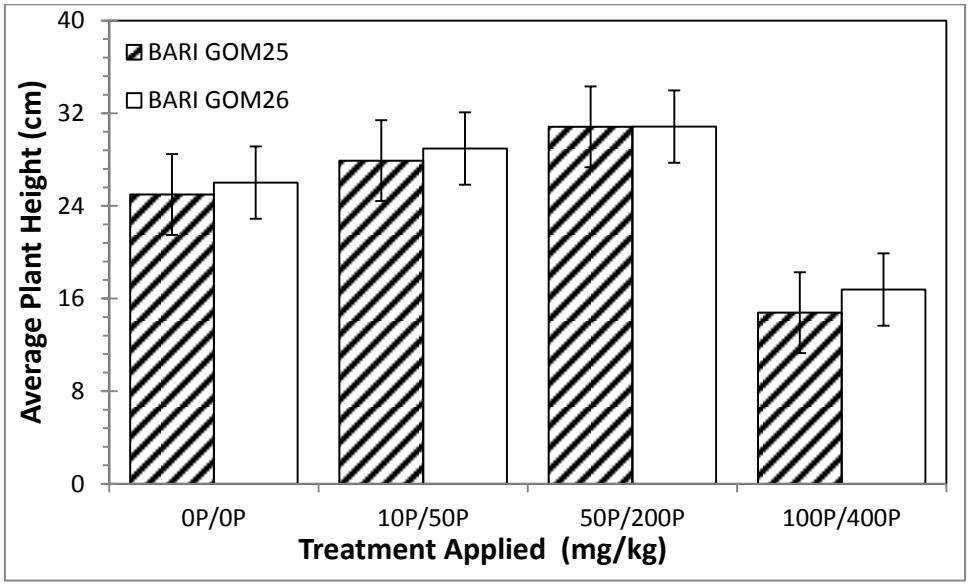

Figure 8-1: Effect of P application on average plant height of the wheat seedlings that was grown in various level of P for 28 days.

8.4.2 Shoot dry weight

Like plant height, the shoot biomass showed similar trend under different P applications. The results showed for the variety BARI GOM 25 that the maximum shoot biomass (0.85 gm/pot) was recorded in Treatment C (50P/200P mg/kg), while minimum (0.25 gm/pot) was found in Treatment D (100P/400P mg/kg). Again, the results showed for the variety BARI GOM 26 that the maximum shoot biomass (0.87 gm/pot) was recorded in Treatment C (50P/200P mg/kg), while it was minimum (0.26 gm/pot) in Treatment D (100P/400P mg/kg). 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 phosphorus application, but at high level P resulted in minimum shoot biomass (Figure 8-2).

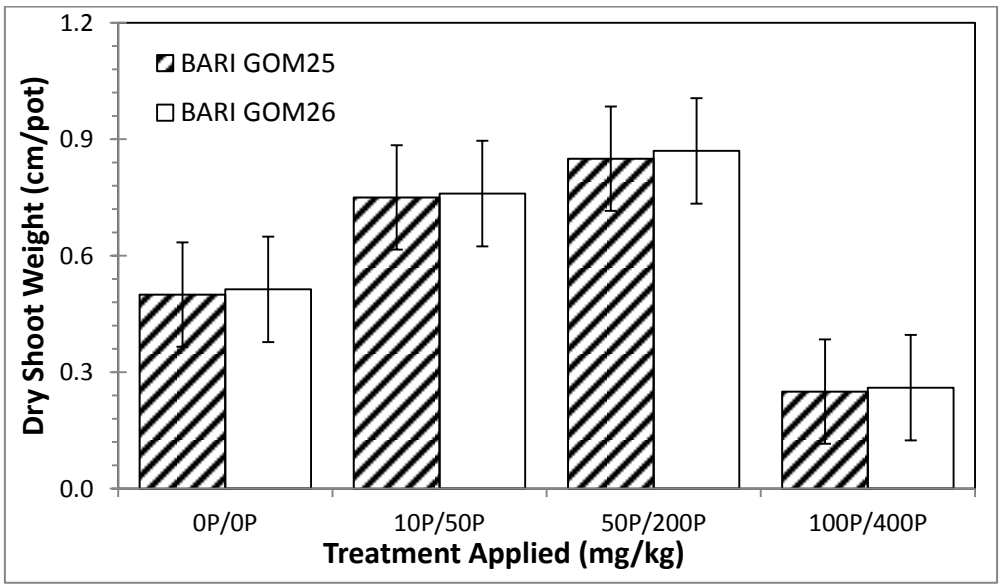

Figure 8-2: Effect of P application on dry shoot weight of the wheat seedlings that was grown in various level of P for 28 days.

8.4.3 Root dry weight

Total root biomass varied among the treatments. Total root biomass of BARI GOM 26 in Treatment C on Compartment II (200 mg/kg P in acidic soil) was the highest (0.57gm/pot) and the lowest in Treatment D-II (400 mg/kg P in acidic soil) (0.17 gm/pot), followed by gradual increase in the Treatment A-I (0 mg/kg P in alkaline soil) (0.26 gm/pot), Treatment A-II (0 mg/kg P in acidic soil) (0.29 gm/pot), Treatment B-I (10 mg/kg P in alkaline soil) (0.36 gm/pot) , Treatment C-I (50 mg/kg P in alkaline soil) (0.41 gm/pot), Treatment B-II (50 mg/kg P in acidic soil) (0.46 gm/pot) and Treatment D-I (100 mg/kg P in alkaline soil) (0.51 gm/pot) (Figure 8-3). Again, for BARI GOM 25 total root biomass was the highest in Treatment C on Compartment II (200 mg/kg P in acidic soil) (0.55gm/pot) and was the lowest in Treatment D-II (400 mg/kg P in acidic soil) (0.15 gm/pot), followed by gradual increase in the Treatment A-I (0 mg/kg P in alkaline soil) (0.25 gm/pot), Treatment A-II (0 mg/kg P in acidic soil) (0.28 gm/pot), Treatment B-I (10 mg/kg P in alkaline soil) (0.35 gm/pot), Treatment C-I (50 mg/kg P in alkaline soil) (0.40 gm/pot), Treatment B-II (50 mg/kg P in acidic soil) (0.45 gm/pot) and Treatment D-I (100 mg/kg P in alkaline soil) (0.50 gm/pot). Similar to shoot dry weight, root biomass was significantly ($P \leq 0.001$) affected among all the various P application on wheat plant. But, the root biomass did not significantly ($P > 0.05$) differ between varieties of wheat plant.

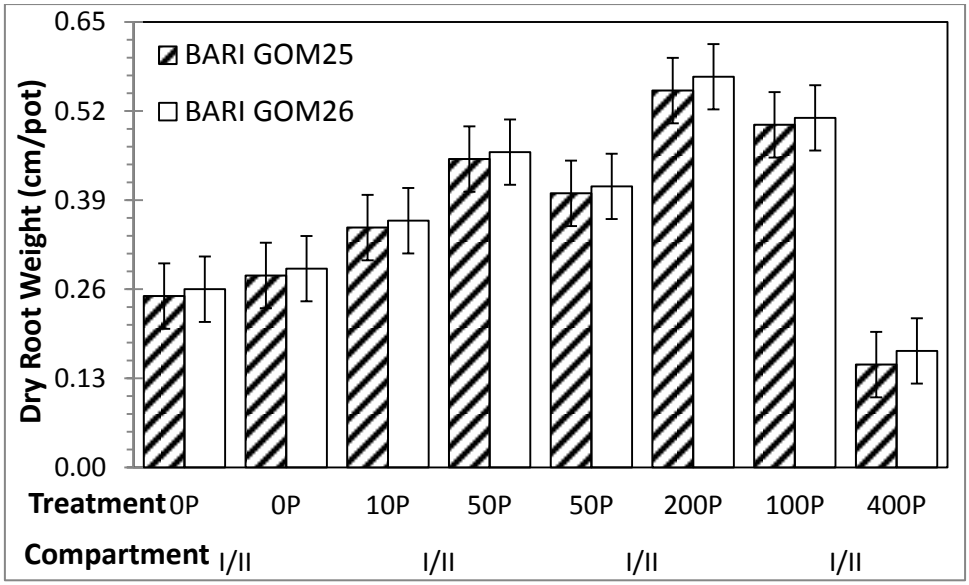

Figure 8-3: Effect of P application on dry root weight of the wheat seedlings that was grown in various level of P for 28 days.

8.4.4 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) (2.74 gm/kg) and Treatment A (0P/0P mg/kg) (0.41 gm/kg) respectively, but intermediates in Treatments B (10P/50P mg/kg) (1.43 gm/kg) and Treatment D (100P/400P mg/kg) (0.71 gm/kg) (Figure 8-4). Again, for BARI GOM 25 total soot P concentration was the highest and the lowest in Treatment C (50P/200P mg/kg) (2.52 gm/kg) and Treatment A (0P/0P mg/kg) (0.40 gm/kg) respectively, but intermediates in Treatments B (10P/50P mg/kg) (1.30 gm/kg) and Treatment D (100P/400P mg/kg) (0.63 gm/kg). The shoot P concentration of BARI

GOM 25 and BARI GOM 26 were dependent on the treatments. However, the two varieties had similar responses on shoot P concentration in the different treatments.

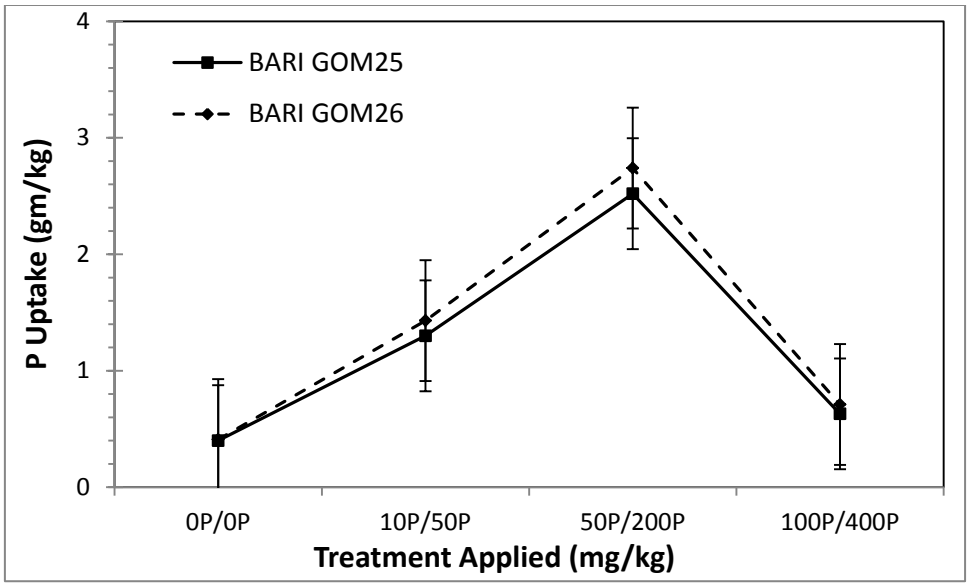

Figure 8-4: Effect of P application on P uptake of wheat shoot in various level of P for 28 days.

8.4.5 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 P in acidic soil) (2.77 gm/kg) and Treatment D-II (400 mg/kg P in acidic soil) (0.19 gm/plant) respectively, followed by gradual increase in Treatment A-I (0 mg/kg P in alkaline soil) (0.29 gm/plant) , Treatment A-II (0 mg/kg P in acidic soil) (0.31 gm/plant), Treatment B-I (10 mg/kg P in alkaline soil) (0.53 gm/plant) , Treatment B-II (50 mg/kg P in acidic soil) (0.68 gm/plant), Treatments C-I (50 mg/kg P in alkaline soil) (1.63 gm/plant) and Treatment D-I (100 mg/kg P in

alkaline soil) (2.22 gm/plant) (Figure 8-5). Again, for BARI GOM 25 total root P concentration was the highest and the lowest in Treatment C in Compartment II (200 mg/kg P in acidic soil) (2.61 gm/kg) and Treatment D-II (0 mg/kg P in acidic soil) (0.17 gm/plant) respectively, followed by gradual increase in Treatment A-I (0 mg/kg P in alkaline soil) (0.27 gm/plant) , Treatment A-II (0 mg/kg P in acidic soil) (0.28 gm/plant), Treatment B-I (10 mg/kg P in alkaline soil) (0.49 gm/plant) , Treatment B-II (59 mg/kg P in acidic soil) (0.68 gm/plant), Treatments C-I (50 mg/kg P in alkaline soil) (1.45 gm/plant) and Treatment D-I (100 mg/kg P in alkaline soil) (2.07 gm/plant) The root P concentration of BARI GOM 25 and BARI GOM 26 were dependent on the treatments. However, the two varieties had similar responses on root P concentration in the different treatments.

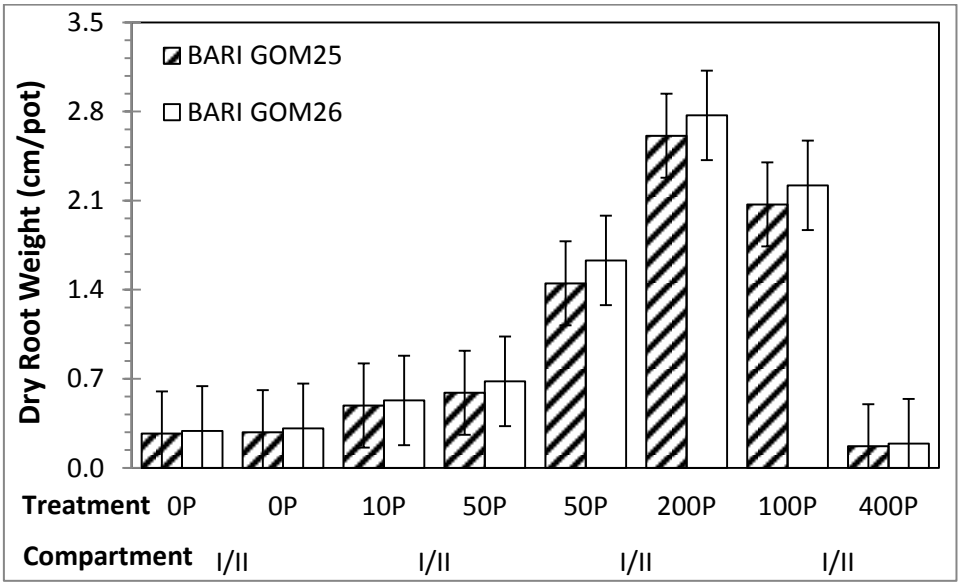

Figure 8-5: Effect of P application on P uptake of wheat root in various level of P for 28 days.

8.4.6 Total P uptake and P distribution

In both varieties of BARI GOM 25 and BARI GOM 26 similar trend in total P uptake were found. The total P uptake by plant was significantly high in Treatment C (50P/200P mg/kg) from other treatments in both varieties. However, total P uptake was more than six times greater in Treatment C (50P/200P mg/kg) than control Treatment A (0P/0P mg/kg). The total P uptake was greater in BARI GOM 26 than that of BARI GOM 25 in all treatments. Total P uptake in BARI GOM 26 of Treatment C (50P/200P mg/kg) and Treatment A (0P/0P mg/kg) were the highest (7.14 gm/kg) and the lowest (1.01 gm/kg), but intermediates in Treatment B (10P/50P mg/kg) (2.64 gm/kg) and Treatment D (100P/400P mg/kg) (3.12 gm/kg) (Figure 8-6). Again, for BARI GOM 25 total P uptake in Treatment C (50P/200P mg/kg) and Treatment A (0P/0P mg/kg) were the highest (6.58 gm/kg) and the lowest (0.95 gm/kg), but intermediates in Treatments B (10P/50P mg/kg) (2.38 gm/kg) and Treatment D (100P/400P mg/kg) (2.87 gm/kg).

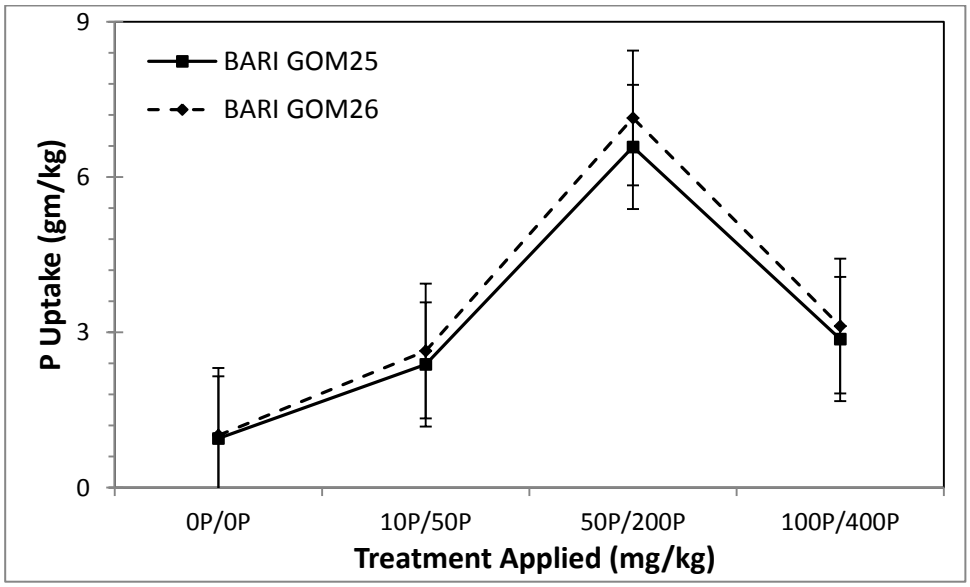

Figure 8-6: Effect of P application on P uptake of wheat plant in various level of P for 28 days.

8.5 Discussion

8.5.1 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 *et al.*, 2005 and 2004). 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 *et al.*, 2005 and 2004). 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 ($P \leq 0.001$) in this study indicates that the plant growth responses of BARI GOM 25 and BARI GOM 26 seedlings were dependent on the level of added P. In all treatments, there were no significant differences between BARI GOM 25 and BARI GOM 26 seedlings for any growth measurement. Total plant biomass in BARI GOM 26 of Treatment C increased 74.5% (1.85 gm/pot) in comparison with the controlled Treatment A (1.06 gm/pot). Similarly in Treatment B increased 49.1% (1.55 gm/pot) and in Treatment D decreased 11.3% (0.94 gm/pot) in

comparison with Treatment A. Again, for BARI GOM 25 total plant biomass in Treatment C increased 74.7% (1.80 gm/pot) in comparison with the controlled Treatment A (1.03 gm/pot). Similarly in Treatment B increased 50.5% (1.55 gm/pot) and in Treatment D decreased 12.6% (0.90 gm/pot) in comparison with Treatment A. Similar trend was found in shoot biomass and root biomass of both wheat plant varieties in this study (Table 8.4). But, internal biomass distribution in shoot and root was found no common trend among all treatments (Figure 8-7). The shoot biomass was found highest (48.5% of total plant biomass) in Treatment A of BARI GOM 25 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 8-7). 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 GOM 26 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 8.4). The inhibitory effect of increasing the P supply to whole root systems on the development of cluster roots of wheat plant (*Triticum aestivum* L.) is well documented (Qifu *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.

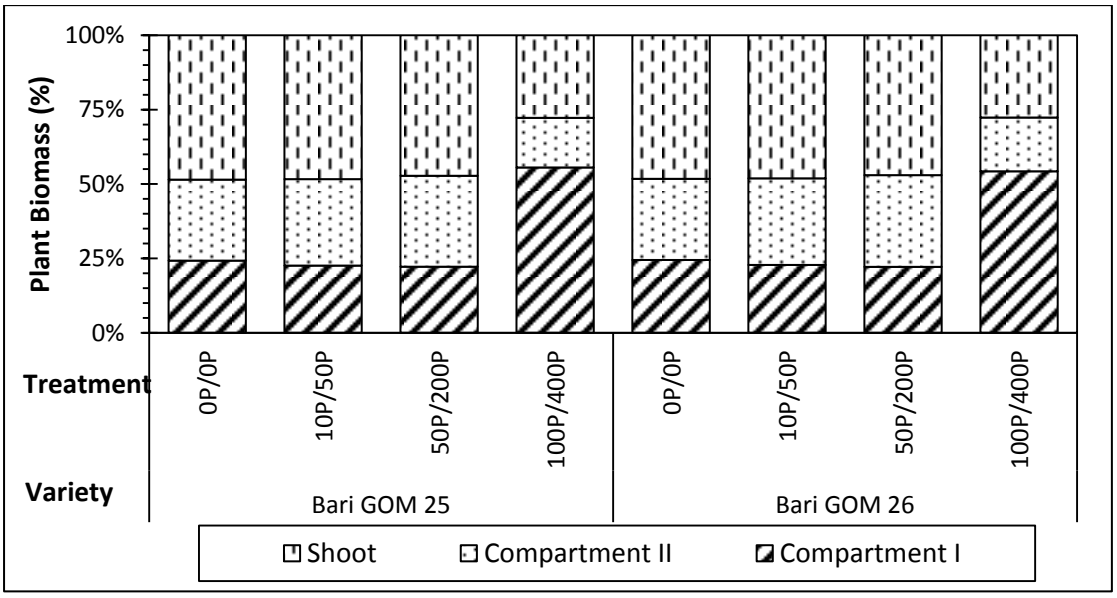

Figure 8-7: The distribution of plant biomass in different plant parts of the split-root system

Table 8.4: 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 /Varieties	Total Plant Biomass (gm /pot)			
	Treatment A	Treatment B	Treatment C	Treatment D
BARI GOM 25	1.03	1.55	1.80	0.90
BARI GOM 26	1.06	1.58	1.85	0.94
Total Biomass (gm/pot) in different plant parts of the split-root system				
BARI GOM 25				
Shoot	0.50	0.75	0.85	0.25
Compartment-I	0.25	0.35	0.40	0.5
Compartment-II	0.28	0.45	0.55	0.15
BARI GOM 26				
Shoot	0.51	0.76	0.87	0.26
Compartment-I	0.26	0.36	0.41	0.51
Compartment-II	0.29	0.46	0.57	0.17
The distribution of Biomass (%) in shoot and roots grown in two separate soil compartments (I and II)				
BARI GOM 25				
Shoot	48.5	48.4	47.2	27.8
Compartment-I	24.3	22.6	22.2	55.6
Compartment II	27.2	29.0	30.6	16.7
BARI GOM 26				
Shoot	48.3	48.1	47.0	27.7
Compartment I	24.5	22.8	22.2	54.3
Compartment II	27.3	29.1	30.8	18.1

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 8.5). 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 Qifu *et al.*, 2011.

Table 8.5: Root biomass, shoot biomass, and root/shoot ratio of two wheat varieties across different P applications

Varieties	P rate (mg/kg)	Treatment	Biomass Production (mg/pot)		Root-shoot ratio
			Shoot	Root	
BARI GOM 25	0P/0P	A	0.50	0.53	1.06
	10P/50P	B	0.75	0.80	1.07
	50P/200P	C	0.85	0.95	1.12
	100P/400P	D	0.25	0.65	2.60
BARI GOM 26	0P/0P	A	0.51	0.55	1.07
	10P/50P	B	0.76	0.82	1.08
	50P/200P	C	0.87	0.98	1.13
	100P/400P	D	0.26	0.68	2.62

8.5.2 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 a 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 ($P \leq 0.001$) affected among all the various P applications on wheat

plant. Again, similar trend in total P uptake were found in both varieties of BARI GOM 25 and BARI GOM 26. Total plant P fixation in BARI GOM 25 of Treatment C increased about 7 times (6.58 gm/kg) in comparison with the controlled Treatment A (0.95 gm/kg). Similarly in Treatment B and Treatment D total plant P fixation increased about 2.5 times (2.38 gm/kg) and 3 times (2.87 gm/kg) respectively in comparison with Treatment A. Again, for BARI GOM 26, total plant biomass in Treatment C increased 7 times (7.14 gm/kg) in comparison with the controlled Treatment A (1.01 gm/kg). Similarly in Treatment B and Treatment D the total plant P fixation increased about 2.5 times (2.64 gm/kg) and 3 times (3.12 gm/kg) 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 8.6); while internal P uptake by shoot and root was found irregular pattern among all treatments (Figure 8-8). 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 it 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 8.6). 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 of 28.4%, 22.0% and 20.6% 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 39.7% of total plant P uptake and in Treatment A, Treatment B and Treatment D the percentages were found in decreasing order of 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 8-8). 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.

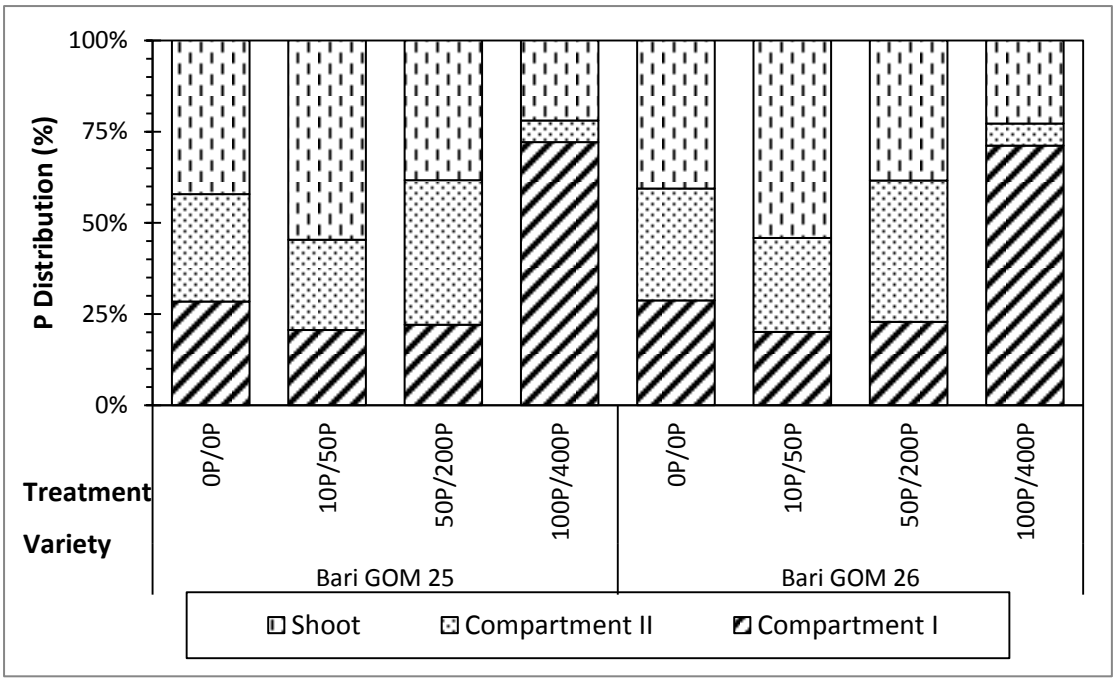

Figure 8-8: The P distribution in different plant parts of the split-root system

Table 8.6: Total P uptake in different plant parts of the split-root system and distribution of P in shoot and root of two separate compartments.

Plant parts /Varieties	Total P uptake (gm /kg)			
	Treatment A	Treatment B	Treatment C	Treatment C
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 (gm/kg) in different plant parts of the split-root system				
BARI GOM 25				
Shoot	0.40	1.30	2.52	0.63
Compartment-I	0.27	0.49	1.45	2.07
Compartment-II	0.28	0.59	2.61	0.17
BARI GOM 26				
Shoot	0.41	1.43	2.74	0.71
Compartment-I	0.29	0.53	1.63	2.22
Compartment-II	0.31	0.68	2.77	0.19
The distribution of P (%) in shoot and roots grown in two separate soil compartments (I and II)				
BARI GOM 25				
Shoot	42.1	54.6	38.3	22.0
Compartment-I	28.4	20.6	22.0	72.1
Compartment II	29.5	24.8	39.7	5.90
BARI GOM 26				
Shoot	40.6	54.2	38.4	22.8
Compartment I	28.7	20.1	22.8	71.2
Compartment II	30.7	25.8	38.8	6.10

Several researchers described a picture of patterns of inorganic P movement in whole plants (Mimura *et al.*, 1996; Jeschke *et al.*, 1997). 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 to 400 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 with, e.g. barley (Drew & Saker 1984), subterranean clover (Scott & Robson 1991), tomato (Burleigh & 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 those Compartment II. Studies with barley (Greenway & Gunn

1966; Clarkson & 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.

8.6 Conclusion

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. This study reveals P uptake and P translocation in split root system of the wheat plant. These findings indicate 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. Again, no varietal difference is found in various experiments. Further, 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, resulting in increased P concentrations in the roots of wheat plants. Finally, the results indicate that split root system and elevated P applications were regulated systematically by the P status of the shoot, and the P fixations in the roots had significant influence on growth and P translocation in wheat plant (*Triticum aestivum* L.).

Chapter 9

General Discussion

Chapter 9

9. General Discussion

9.1 General discussion

The focus of this thesis has been to understand the mechanisms involved in the utilization of phosphorus by wheat plant that means, P uptake, distribution and transport within wheat plants. In order to understand the P utilization mechanism in two recently BARI developed wheat varieties namely BARI GOM 25 and BARI GOM 26 were used for their responses to the treatments. The major findings are i) Phosphorus use efficiency in both acidic and alkaline soil conditions; ii) Growth responses of wheat plant in different root system under both acidic and alkaline soil conditions; iii) Phosphorus acquisition by wheat plant in split-root systems under both acidic and alkaline soil conditions; and iv) Phosphorus dynamics in wheat plant in split-root systems. A number of additional features relating to these experiments have been discussed in this chapter.

9.1.1 Phosphorus use efficiency in different soil conditions

In general, plants grow better when partially soluble phosphate is applied in comparison to the soluble P source. Due to the low mobility of P in soils, root growth is very important for P acquisition (Föhse *et al.*, 1988; Watt and Evans, 2003; Lynch, 2007). For plants, phosphate uptake from the soil is often difficult: in alkaline soils, P is bound to calcium and in acidic soils, P is usually bound to aluminium and/or to iron (Kochian *et al.*, 2004). In addition, organic material present in the soil (e.g. from

manure or from crop debris) can also bind phosphate, particularly in phytate (inositol compounds). Likewise, Rubio *et al.*, (2003) reported the effect of pH change over P stability and indicating the soil pH influences on its availability. Soil pH influences the charge of the P species in solution as well as the charge of the adsorbing particles in soils. When pH rises, an increase in the negative charge is produced thereby decreasing P adsorption; but, at the same time, an increase in HPO_4^{2-} concentration is produced which has a much greater affinity for reactive soil surfaces which in turn increases P adsorption (Schachtman *et al.*, 1998). Whether a change in soil pH will increase or decrease P in soil solution sometimes depends on the dominant effect (Whitelaw, 2000). Therefore, below pH 6.0, most P will be present as the monovalent $H_2PO_4^-$ species, whereas H_3PO_4 and HPO_4^{2-} will be present only in minor proportions. Most studies on the pH dependence of P uptake in higher plants have revealed that uptake rates are highest between pH 5.0 and 6.0, where $H_2PO_4^-$ dominates (Ullrich-Eberius *et al.*, 1984: Furihata *et al.*, 1992), suggests that P is taken up as the monovalent form. The study was conducted in different soil types - both acidic soil and alkaline soil and P doses were applied directly to the soil. The maximum PUE of 7.75% was observed at 60 mg/kg P rate for variety BARI GOM 25 in acidic soil and it decreased significantly at higher P rates. While in alkaline soil, similar trend was observed having the maximum PUE of 7.32% at 60 mg/kg P rate. Similarly, the maximum PUE of 7.95% was observed at 60 mg/kg P rate for variety BARI GOM 26 in acidic soil and it decreased significantly at higher P rates. While in alkaline soil, similar trend was observed having the maximum PUE of 7.53% at 60 mg/kg P rate. Results indicate that plant growth increased significantly with the use of P. Again, , PUE was recorded higher in acidic soil among all P applications in comparison with alkaline soil, which means plants have more favorable condition for P utilization on

plant growth in acidic soil. However, it is clear from Figure 9-1 that elevated P application has significant influence the PUE of wheat plant.

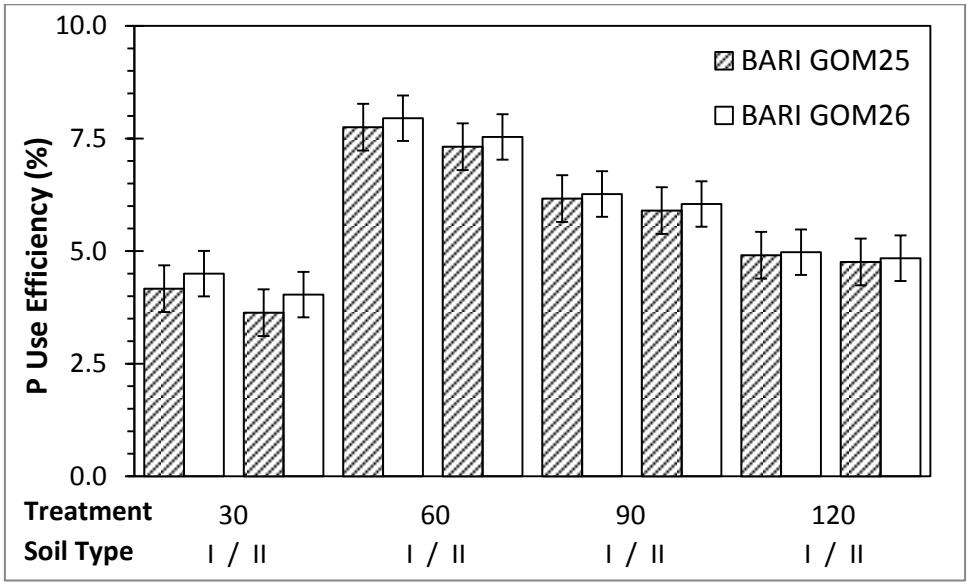

Figure 9-1: P use efficiency of two wheat varieties across different P levels

9.1.2 Growth Responses in different root system under different soil conditions

Plants typically respond to P limitation by reducing total plant biomass, and diverting resources disproportionately towards root growth (Zhu *et al.*, 2005 and 2004). 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 P condition, plants that proliferate roots into these upper layers outperform varieties with deeper root systems (Zhu *et al.*, 2005 and 2004). Root proliferation and greater uptake per unit of root in the nutrient-rich zones are often considered to be compensatory responses. To investigate growth response of recently BARI released wheat varieties under elevated

P applied condition of different experiments, all growth measurements, including root biomass, plant height and shoot biomass measures were taken. The highly significant treatment interaction for plant growth ($P \leq 0.001$) in this study indicates that the plant growth responses of BARI GOM 25 and BARI GOM 26 seedlings were dependent on the level of added P. In all treatments, there were no significant differences between BARI GOM 25 and BARI GOM 26 seedlings for any growth measurement. The highly significant soil interaction for plant growth ($P \leq 0.001$) in this study indicates that the plant growth responses of BARI GOM 25 and BARI GOM 26 seedlings were dependent on the level of added P in different soil condition.

Analysis of variance reveals that phosphorus application has significant effect on plant height. It also increased with the increasing level of P application. From the plant growth experiments, the maximum plant height in BARI GOM 26 (34.93 mm in acidic soil and 32.8 mm in alkaline soil) were found in Treatment T5, while in BARI GOM 25 (34.7 mm in acidic soil and 31.9 mm in alkaline soil) were recorded in Treatment T5. In split-root experiments, the maximum plant height in BARI GOM 26 of Treatment C increased 35.89 mm in acidic soil and 30.99 mm in alkaline soil in comparison with the controlled Treatment A. Similarly in BARI GOM 25 the plant height in Treatment C increased 34.75 mm in acidic soil and 30.76 mm in alkaline soil. Again, for combined soil experiment the maximum plant height (30.85 mm in BARI GOM 26 and 30.83 mm in BARI GOM 25) were recorded in Treatment C. Thus, plant height was significantly ($P \leq 0.001$) affected among all the various P application and variety of wheat plant in different plant root system. The results are in line with the accordance of Holloway *et al.*, 2001; Khan *et al.*, 2010; Maqbool *et al.*, 2012 who also reported the similar findings.

All experiments results showed that elevated P application increased biomass production. Compared to the control, application of P significantly improved biomass production, especially with higher P applications. In plant growth experiments, the highest total plant biomass in BARI GOM 26 was obtained (1.54 gm/plant in acidic soil and 1.46 gm/plant in alkaline soil) in Treatment T5. Similarly in BARI GOM 25 of Treatment T5 the highest total plant biomass was obtained (1.51 gm/plant in acidic soil and 1.41 gm/plant in alkaline soil). While in split root experiments, the highest total plant biomass in BARI GOM 26 was found (2.25 gm/plant in acidic soil and 1.66 gm/plant in alkaline soil) in Treatment C. Again, for BARI GOM 25 the highest total plant biomass was found (2.22 gm/plant in acidic soil and 1.61 gm/plant in alkaline soil) in Treatment C. In case of combined soil experiment the highest total plant biomass in BARI GOM 26 was found 1.85 gm/plant in Treatment C. Again, for BARI GOM 25 highest total plant biomass was found 1.80 gm/plant in Treatment C. Waitrak (2013); de Figueiredo *et al.*, (2012) reported similar findings on field application.

The shoot biomass was also significantly ($P \leq 0.001$) affected among all the various P applications on wheat plant. In plant growth experiments, for the variety BARI GOM 25, the maximum shoot biomass (0.85 gm/plant in acidic soil and 0.81 gm/plant in alkaline soil) was recorded in Treatment T5 (120 gm/kg P). Again, for the variety BARI GOM 26 the maximum shoot biomass (0.87 gm/plant in acidic soil and 0.83 gm/plant in alkaline soil) was obtained in Treatment T5 (120 gm/kg P). While in split root experiments, the maximum shoot biomass in BARI GOM 25 (0.9 gm/plant in

acidic soil and 0.60 gm/plant in alkaline soil) was recorded in Treatment C. Similarly, for BARI GOM 26 the maximum shoot biomass was recorded (0.91 gm/plant in acidic soil and 0.62 gm/plant in alkaline soil) in Treatment C. In case of combined soil experiment the maximum shoot biomass in BARI GOM 25 was found 0.87 gm/plant in Treatment C. Again, for BARI GOM 26 maximum shoot biomass was found 0.85 gm/plant in Treatment C. Similar to shoot biomass, root biomass was also significantly ($P \leq 0.001$) affected among all the various P application on wheat plant. The root biomass was significantly ($P \leq 0.001$) affected between both acidic and alkaline soil. In plant growth experiment, the total root biomass in BARI GOM 26 were the highest in Treatment T5 (0.67 gm/plant in acidic soil and 0.63gm/plant in alkaline soil) and the lowest in Treatment T1 (control) (0.32 gm/plant in acidic soil and 0.26 gm/plant in alkaline soil) respectively. Again, for BARI GOM 25 total root biomass was the highest in Treatment T5 (0.65 gm/plant in acidic soil and 0.60 gm/plant in alkaline soil) and the lowest in Treatment T1 (0.30 gm/plant in acidic soil and 0.25 gm/plant in alkaline soil) respectively. While in split root experiments, the maximum root biomass in BARI GOM 25 (0.70 gm/plant in acidic soil and 0.55 gm/plant in alkaline soil) was recorded in Treatment C - Compartment II. Similarly, for BARI GOM 26 the maximum root biomass was recorded (0.71 gm/plant in acidic soil and 0.57 gm/plant in alkaline soil) in Treatment C in Compartment II. In case of combined soil experiment the maximum root biomass in BARI GOM 25 was found 0.55 gm/plant in Treatment C in Compartment II. Again, for BARI GOM 26 maximum root biomass was found 0.57 gm/plant in Treatment C in Compartment II. The inhibitory effect of increasing the P supply to whole root systems on the development of cluster roots of wheat plant (*Triticum aestivum* L.) is well documented (Qifu *et al.*, 2008, Pedas *et al.*, 2011, Iqbal, 2014). From the experiments

in different soil and different root system, the total root and shoot biomass increased due to elevated P supply that means elevated P application directly interferes with shoot and root growth.

Table 9.1: Root/shoot ratio of two wheat varieties across different P levels

Varieties	Treatment	P rate (mg/kg)	Acidic Soil	Alkaline Soil
BARI GOM 25	T1	0	0.66	0.66
	T2	30	0.73	0.70
	T3	60	0.73	0.70
	T4	90	0.75	0.73
	T5	120	0.77	0.74
BARI GOM 26	T1	0	0.69	0.64
	T2	30	0.73	0.69
	T3	60	0.74	0.71
	T4	90	0.75	0.73
	T5	120	0.78	0.76
Split-root system				
Varieties	**Treatment**	**P rate (mg/kg)**	**Acidic Soil**	**Alkaline Soil**	**Mixed soil**
BARI GOM 25	T1	0P/0P	1.13	1.17	1.06
	T2	10P/50P	1.31	1.37	1.07
	T3	50P/200P	1.45	1.67	1.12
	T4	100P/400P	-	-	2.60
BARI GOM 26	T1	0P/0P	1.17	1.18	1.07
	T2	10P/50P	1.31	1.40	1.08
	T3	50P/200P	1.46	1.67	1.13
	T4	100P/400P	-	-	2.62

The root-shoot ratio is an important factor to understand growth responses of plants under elevated P applications in different soil condition. Root-shoot ratio is one of several ratios, which give estimates of the distribution of dry matter between the different plant organs (Boutraa *et al.*, 2010). It is a measure of the distribution of dry matter between the root and the shoot systems and it is a good indicator for the effects

on root and shoots dry weights. The results showed that root-shoot ratio was significantly ($P > 0.05$) affected among all the various P applications on wheat plant. Comparison of root: shoot ratio of different treatments showed an increase with the increasing P application in both varieties of BARI released wheat plants and also in both soil conditions. But, in split root system root-shoot ratio increased significantly in comparison with the plant growth experiments, which means split root system directly interferes with root growth. 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 Qifu *et al.*, 2011.

9.1.3 P acquisition by wheat plant in different root system under different soil conditions

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 wheat accessions were grown in acid and alkaline soils for 28 days. At the end of the growing period, plants were harvested to measure biomass and P-fixation for each accession. After harvesting, the shoot P fixation amounts of the different treatments were compared. At low level of P supply, shoot P uptake significantly increased in comparison with the controlled treatment. Similar results were observed in different genotypes of wheat (Ahmad *et al.*, 2013; Hu *et al.*, 2014). The shoot P fixation was significantly ($P \leq 0.001$) affected among all the various P application on wheat plant. In plant growth experiment, the total shoot P fixation in BARI GOM 26 were the

highest in Treatment T5 (4.53 gm/kg in acidic soil and 4.49 gm/kg in alkaline soil) and the lowest in Treatment T1 (control) (0.43 gm/kg in acidic soil and 0.41 gm/kg in alkaline soil) respectively. Again, for BARI GOM 25 total shoot P fixation was the highest in TreatmentT5 (4.49 gm/kg in acidic soil and 4.41 gm/kg in alkaline soil) and the lowest in Treatment T1 (0.41 gm/kg in acidic soil and 0.40 gm/kg in alkaline soil) respectively. While in split root experiments, the highest shoot P fixation in BARI GOM 25 (4.23 gm/kg in acidic soil and 4.02 gm/kg in alkaline soil) was recorded in Treatment C. Similarly, for BARI GOM 26 the highest shoot P fixation was recorded (4.39 gm/kg in acidic soil and 4.27 gm/kg in alkaline soil) in Treatment C. In case of combined soil experiment the highest shoot P fixation in BARI GOM 25 was found 2.52 gm/kg in Treatment C. Again, for BARI GOM 26 it was found 2.74 gm/kg in Treatment C. This is in line with the suggestion of Cavagnaro *et al.*, (2003) that, P uptake rate by plant shoot was significantly high at low P concentration. At low level of P supply, root P uptake was primarily found to increase in comparison with the controlled treatment. At elevated amounts of accessible P, there was no noteworthy contrast among all treatments. Comparative results were reported in different genotypes of wheat (Hu *et al.*, 2014).

Similar to shoot P fixation, root P fixation was also significantly ($P \leq 0.001$) affected among all the various P application on wheat plant. In plant growth experiment, the total root P fixation in BARI GOM 26 were the highest in Treatment T5 (2.11 gm/kg in acidic soil and 2.01 gm/kg in alkaline soil) and the lowest in Treatment T1 (control) (0.32 gm/kg in acidic soil and 0.31 gm/kg in alkaline soil) respectively. Again, for BARI GOM 25 total shoot P fixation was the highest in Treatment T5 (2.07 gm/kg in acidic soil and 1.99 gm/kg in alkaline soil) and the lowest in Treatment T1 (0.28

gm/kg in acidic soil and 0.27 gm/kg in alkaline soil) respectively. While in split root experiments, the highest shoot P fixation in BARI GOM 25 (2.97 gm/kg in acidic soil and 2.89 gm/kg in alkaline soil) was recorded in Treatment C - Compartment II. Similarly, for BARI GOM 26 the highest shoot P fixation was recorded (3.12 gm/kg in acidic soil and 2.97 gm/kg in alkaline soil) in Treatment C – Compartment II. In case of combined soil experiment the highest shoot P fixation in BARI GOM 25 was found 1.67 gm/kg in Treatment C – Compartment II. Again, for BARI GOM 26 it was found 1.82 gm/kg in Treatment C – Compartment II. This pattern of P fixation in plant root and shoot among both varieties and both soil conditions are indicated elevated P supply directly interferes with shoot-root P status.

The plant P fixation measures of the various treatments were investigated. Similar to the shoot and root phosphorus fixation pattern total plant phosphorus fixation results were compared with different treatments. The amount of P fixation in acidic soil was higher than in alkaline soil. Similar pattern on p fixation was obtained in both plant growth experiments and split root experiments. In case of combined soil experiments, p fixation in acidic soil was higher than in alkaline soil. This pattern of P fixation in wheat plant among all root system and both soil conditions indicated that elevated P supply directly interferes with shoot-root P status.

9.1.4 P translocation in wheat plant in different root systems

My findings suggested that P is translocated within root and shoot part although there was elevated P supplied within split-root system in both acidic and alkaline 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 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 re-translocation 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 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 with, e.g. barley (Drew & Saker 1984), subterranean clover (Scott & Robson 1991), tomato (Burleigh & 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 (Marschner *et al.*, 1987) demonstrate that P retranslocated in the phloem sap can result in an increased root P concentrations. In comparison with plant growth experiments to split root experiments, P fixation was

found higher in lower P applied compartments in split root system. On the other hand, P fixation was found lower in higher P applied compartments in split root system. In split-root plants, very high P supplies (200 mg P/kg KH_2PO_4) to just one crown root of wheat plants significantly increased the P concentration of Compartment-I roots in respect of 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 Compartment II. Both BARI released wheat varieties showed similar pattern under different soil conditions (Chapter 6 and chapter 7). Again, in Chapter 8 experiments on 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; Furihataet *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 & Gunn 1966; Clarkson & 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 in acidic soil probably provided more P for shoot unloading of P and for P assimilation in the controlled roots, resulting increased P concentrations in the roots of wheat plants. In contrast, the split-root technique in acidic soil probably provides a more stable supply of P at a lower concentration.

9.1.5 Seedlings is a tool and its effect on P utilization efficiency on both acidic and alkaline soil

Seedlings are the most sensitive stage of growth of plants to both acidic and alkaline soils. For example, the deleterious effect of H^+ and OH^- ion on the growth of wheat was noticeable only in the seedling stage. At the seedling stage, wheat is sensitive to H^+ and OH^- ion, but looses of some of this sensitivity during more advanced stages of growth (Thawornwong and Diest 1974). Conducting the experiments in this thesis with seedlings in the normal environment conditions in the air had several advantages. First, the seedling stage of growth is sensitive to both H^+ and OH^- ion toxicity and so differences between wheat varieties are likely to be readily detectable. Second, it was possible to get rapid results from the seedling experiments. This meant that the seedling experiment could be repeated to confirm the results. Third, it was possible to conduct short-term experiments where the seedlings relied on nutrient reserves in the seed. This enabled any confounding effects from the addition of basal nutrients to be avoided. An example could be the addition of $SO_4^{2-}S$, which reacts with Al^{3+} and reduces the toxic Al^{3+} concentrations (Alva *et al.*, 1991). The experiments revealed that seedlings could survive for at least 2 weeks when grown in soil system from their existing seed reserves under controlled environment conditions (Chapters 4 and 5). On the other hand, seedling studies have the disadvantage is that they respond somewhat differently to mature plants under field conditions. Thus, the critical level of olsen extractable P for P toxicity is less for wheat plants grown in field then for seedlings in my experiments. This is because field conditions are different to the controlled environment conditions. In the field, plants are frequently exposed to varying degrees of water deficit stress, whereas in controlled environment condition

the seedlings do not face any moisture stress. Even with small roots, the seedlings were able to take up moisture. This is unlikely to happen in the field. Therefore, seedling growth and particularly their shoot growth, is less sensitive to soil P concentration in the experiments outlined in this thesis, than to the similar P concentration in soils in the field.

Chapter 10

Conclusion

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10. Conclusion

10.1 General conclusion

In conclusion, from experimental perspectives, this results of our studies we carried out are obviously coherent with one another providing new useful information about the mechanism involved in the utilization of P by wheat plant i.e. P uptake, distribution and transport in wheat plant under different soil conditions (acidic and alkaline soils). The plant growth experiments (Chapter 4 and Chapter 5) reveal that the elevated P uptake plays a vital role in the development of wheat plants both in acidic and alkaline soil conditions and the findings from the experiments clearly suggest that the added soluble P is liable to increase the absorption of nutrients from the soil solution and thus, added P is highly effective and efficient both for increasing root-shoot development and P acquisition. The two varieties namely BARI GOM 25 and BARI GOM 26 responded similarly in all the experiments under different types of soil conditions. Since PUE was recorded higher in acidic soil among all the P applications compared with alkaline soil, it is definitely understood that wheat plants find more favorable and sustainable conditions for P utilizations on plant growth in acidic soil.

The highly significant treatment (T) interaction for wheat plant growth ($P \leq 0.001$) in the study of split root experiments obviously show that the plant growth responses of wheat seedlings were mainly dependent on the level of added P. Plant height, plant

biomass, root-shoot P acquisition etc. increased regularly with increasing P in both acidic and alkaline soils. Plant growth responses under different P treatments in each separate compartment, shoot dry biomass and dry root biomass were found very significant, while internal biomass distribution in shoot and root showed a different trend among all treatments. Finally the percentage distribution differences in the total root and shoot dry biomass among the three P treatments are due to the elevated P supply which directly interferes with shoot and root growth.

The experimental findings about P uptake and P translocation in split root system of the wheat plant under different soil conditions clearly indicate that the added soluble P increases the absorption of nutrients from the soil solution in both acidic soil and alkaline soil. The shoot and root P uptake followed an increasing trend with increasing P application under different soil conditions. Shoot and root P uptake were significantly ($P \leq 0.001$) affected among all the various P applications. Again, internal distribution of P uptake by shoot and root was found almost similar pattern among all treatments in both acidic soil and alkaline soil. But, the percentage distribution differences in the total root P uptake and shoot P uptake of total plant P uptake among all the treatments of various experiments are of different trend due to elevated P supply which directly interferes with shoot root P status in split root system. The results exhibiting higher P concentration in the shoot of wheat plants are obviously due to more supply of P for shoot P unloading which results in an increased P concentration in the roots of wheat plants. Again, the percentage differences between the P fixations of roots of Compartment-I and Compartment II were much lower in all experiments, indicating wheat plants are strong enough to translocate P from the root in Compartment I to that of Compartment II. Obviously, the elevated P plays a

significant role in the phosphorus translocation within the wheat plants in split root system in both acidic and alkaline soil condition. Finally, the results indicate that the split root system and elevated P applications are regulated systematically by the P status of the shoot, and the P uptakes in the roots have significant influence on growth and P translocation in wheat plant (*Triticum aestivum* L.).

In summary, P uptake by plant roots from the added P results in greater plant biomass production in both acidic soil and alkaline soil conditions. Elevated P applications resulted higher concentration in the shoot of wheat plants probably provides 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. Split root system and elevated P applications were regulated systematically by the P status of the shoot and the P status in the roots had significant influence on growth and P translocation in wheat plant. Finally, application of P nutrients to acidic soil (in comparison with alkaline soil) is a better option for optimum crop (both BARI GOM 25 and BARI GOM 26) production.

10.2 Recommendation for further research

- Some new technologies such as synchrotron X-ray can be used to detect if discrete phase of phosphates is found in the P amendment in different soils
- Effect of frequent applications of P-rich plant residues on P and micronutrients availability and wheat growth in P-deficient soils

Chapter 11

References

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11. References

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