Evaluation of Mechanisms of Phosphorus Use Efficiency in Traditional Wheat Cultivars for Sustainable Cropping

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Abstract In order to search for low input alternative wheat cultivars, this study aims to investigate the mechanisms of phosphorous (P) uptake and utilization efficiency of two traditional wheat cultivars as compared to a modern cultivar. The experiment was conducted under semi-controlled conditions using four P supplies. Plant growth and P efficiency parameters were studied at two harvest dates. Plant and soil parameters where measured: shoot dry matter, P concentration in shoot, root length, root diameter, specific root density, root length/shoot weight, shoot growth rate, P shoot demand on roots, P influx, P efficiency ratio, P utilization index, extractable and soil solution P concentrations, and P recovery. Traditional and modern cultivars had similar overall uptake (P influx, and recovery) and utilization efficiency. In terms of specific efficiency traits, traditional cultivars had the superiority in having more root size, higher root shoot ratio, slower shoot growth rate and less shoot demand on roots for P, but were inferior in having thicker roots and less specific root density. Investigating P use efficiency mechanisms could be a useful tool in selection programs to separate plant cultivars to superior and inferior, but using different measures of utilization efficiency parameters could be in some cases misleading.

Keywords: sustainable agriculture, food security, traditional cultivars, marginal land, low input cultivars, phosphorous uptake efficiency, wheat

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1. Introduction

The human population is expanding rapidly, and expected to reach more than 9.5 billion in 2050 [1]. To feed this growing population, a massive increase in crop production is required [2], potentially through increasing area under cultivation and improving yield per unit area [3] using intensive cropping. But as a result of the intensification of agriculture and the introduction of high yielding varieties, the soils of many regions of the world are getting depleted in reserve phosphorous (P) at a faster rate, making P deficiency one of the major constraints to crop production [4]. Phosphorous is a non-renewable resource [5], quantitatively the most important inorganic nutrient for crop productivity after nitrogen and potassium, unless supplied as fertilizer [6]. The availability of P in soil is low as a result of its fixation, being utilized by organisms forming organic P, and by sorption onto iron and aluminum [7]. The recovery of fertilizer P is very low, often below 15% in the first year of application and hardly reaches 50% after 30 years [8]. Although, in view of limited P resources [7] and serious environmental and economic consequences [4,9], a considerate use of P is mandatory to correct P deficiencies to fulfill the requirements of modern cultivars [10]. P application is particularly effective in yield formation [11,12], but in organic farming, where inorganic P fertilizers are not permitted [13], the soil P availability is not easily increased [14]. The associative water scarcity due to global climate change [15] make it more challenging to sustain food security [16], while preserving the ecological and energy-related resources of our earth [2,4]. Therefore, in developing countries, where the proportion of less fertile soil is particularly high, it is difficult to fulfill the nutritional requirements of high yielding crops [17,18].

As one of the possible strategies to sustain land use, it is desirable to search for efficient use of nutrients, to increase the production potential on marginal land [11,12,19]. These nutrient efficient species are able to make use of the not readily available nutrients for other inefficient species [11,20]. Therefore, using alternative crops that differ in their response to nutrient supply is a possibility to meet the increasing global demand, and may be only possible if nutrient efficiency mechanisms are elucidated [12,19,21,22,23]. Nutrient use efficiency involves different mechanisms related to soil and plant that contribute to the variability in uptake and utilization of nutrients by different plants in different soils [12,19,24]. The interpretation of the nutrient efficiency may vary greatly [12,19,20,25,26], and in some cases could be even misleading in the quest for identification of mechanisms for enhanced nutrient acquisition and utilization [12,19,22,23].

Phosphorous supply to plants depends on plant parameters (root size and architecture and its P uptake kinetics) and soil parameters (quantity, availability and mobility of P in soil) [19,23,24,27,28,29]. As plants...
absorb P ion, soil solution P concentration decreases at the root surface, disturbing the P equilibrium in soil, creating a concentration gradient, making the adjacent soil release P from the solid soil phase into solution, and transport nutrient from the bulk of the soil to the root [23,30]. Thus phosphorous uptake by roots from the rhizosphere is affected by desorption of P from soil particle surface, transport of P in the soil solution towards the root surface and inflow of P into root cell [31,32]. A prerequisite of uptake is the contact between plant roots and the nutrients in soil, which is achieved by root growth to the places where nutrients are located and accompanied with the transport of nutrients through the soil to the root surface [30]. Therefore, P-efficient plants develop large root systems to expose large areas of root surface to the soil [28,30]. The combination of root growth and nutrient transport through the soil is the basic requirement for plants to explore the soil for nutrients [33].

Different cultivars of wheat are cultivated in the Palestinian areas, some of them are relatively high yielding modern introduced cultivars such as Anbar, and are usually grown in areas with high rainfall (450-500 mm). While traditional wheat cultivars such as Kahhatat and Noursi are usually considered as low input in terms of nutrients and irrigation and grown in areas with low precipitation (250-350 mm) [34]. In marginal areas where the less fertile areas can be potentially used for agriculture, it is difficult to fulfill the nutritional requirements of high yielding cultivars, therefore the search for low input species or improving their nutrient use efficiency is promising. In order to search for low input alternative crops and to understand factors affecting P uptake efficiency among plant cultivars, this study aims to investigate the influence of P supply on the components of P uptake and utilization efficiency of Palestinian traditional wheat cultivars as compared to a modern introduced wheat cultivar in pot experiment under greenhouse conditions.

2. Methodology

2.1. Experimental Design

A pot experiment was conducted in June 2015 to evaluate P uptake efficiency and P dynamics in the rhizosphere of two traditional Palestinian wheat cultivars (Kahhatat and Noursi) and one introduced wheat cultivar (Anbar). The plants were obtained from the National Agricultural Research Center/ Palestine and grown in a low P status loamy soil, using four levels of P supply in a greenhouse having semi-controlled climatic conditions. Before the experiment, field-moist soil samples were sieved to 2-mm particle size, from which, subsamples of soil were air dried and analyzed for extractable P, exchangeable K, Mg, and pH. Initially, the soil (pH 7.0 by water extraction) contained 16.5 mg/kg calcium acetate lactate (CAL) extractable P, 28 mg/kg CAL-exchangeable K, and 141 mg/kg NH₄-acetate exchangeable Mg. 

Mitscherlich pots (6 L) were filled with 3 kg sand (0 mg/kg CAL-extractable P, 3 mg/kg CAL-exchangeable K, and 1.8 mg/kg NH₄-acetate exchangeable Mg, pH in water was 7.3) and 3 kg loamy soil. Four P levels (0.0, 0.2, 0.5, and 1.0 g/pot) were added as Ca(H₂PO₄)₂·H₂O, resulting in solution P (mg P/L soil solution) content of 0.15, 3.24, 13.54 and 38.51 in consecutive added P levels. The extractable P content (mg P/kg soil) of the soil after adding external P were 8.66, 20.30, 74.74 and 133.33 in respective P supplies (0.0, 0.2, 0.5, and 1.0 g/pot). Other nutrients added per pot were 1.0g N (as NH₄NO₃), 1.5g K (as K₂SO₄), 0.8g Mg (as MgSO₄), micronutrients were added in adequate amount for both species in both soil types (mg/pot: 17.5 B, 2.5 Mo, 8 Cu, 50 Mn, and 40 Zn). Ten wheat seeds were sown per each pot and after germination, the seedlings were reduced to six identical plants in each pot. The treatments were replicated four times. Four additional pots per each P level were left unplanted as control for the measurement of extractable and soil solution P concentrations during the experiment without be affected by wheat cultivars. The planted and the unplanted pots were watered daily to nearly a volumetric soil water content of 35 percent. The experiment was conducted as a completely randomized design.

2.2. Harvesting and Analytical Procedures

The plants were harvested in two harvest times. The plants in one pot of each treatment (wheat cultivar and P level) was harvested in the first harvest after 34 days from sowing, and the rest three pots in each treatment were harvested in the second harvest after 48 days from sowing. At each harvest, the soil in each shoot harvested pot was weighed (moist soil with roots), and then the soil was cut to two similar parts (also accurately weighed). One part of the soil in each pot was sieved to remove the roots and then was sub-sampled for the following measurements: a soil sample to measure the moisture content of the soil (around 100g), a soil sample for measuring soil solution P (around 350g), and finally a soil sample for measuring extractable P (around 100g). The second half of the soil of each harvested pot was put in a sealed plastic bag and kept at 6°C for collecting the roots within 48 hours.

Harvested plants were separated into shoots and roots (half roots per pot were collected). Shoots were measured for fresh and dry weights, then were analyzed for their P, K, Ca, and Mg contents. The roots in half soil of the pot (precisely weighed) were separated from the soil by washing it over a 0.2 mm sieve, then were preserved in a plastic bottle at 6°C to be measured for their fresh weight and length within 24 hours.

2.2.1.Shoot Measurements

At harvest, the dry weight of shoot was determined after drying at 70°C till constant weight. Dried plant materials were grinded to pass a 1.5 mm sieve, of which, after thorough mixing, a sub-sample of 5 g was ball-milled to a fine powder. The plant samples were prepared for P analysis using wet microwave digestion using concentrated tri acid mixture (HNO₃, HClO₄, and H₂SO₄ with a volumetric ratio of 8:2:1). Total P, K, Ca, and Mg contents of the plant material digest was measured using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES; Varian-Vista).
2.2.2. Measurement of Soil Solution and Extractable P Concentration

The column displacement method [35] was used to collect the soil solution in order to determine initial soil solution P concentration. The method permits accurate determination of the unaltered composition of soil solution, in which a sample of moist soil equivalent to 350 g was packed into a plastic column with a pore in its bottom. Filter paper was placed in the bottom of each soil column to avoid soil particles losses during the collection. The samples were allowed to equilibrate for 24 h; then, deionized water was pumped to each column at a rate of 4 ml/h until the soils reached field capacity water content. The displaced solution was collected till 25 ml to insure not to collect diluted solution, and then filtered through a 0.20 µm filter. The collected soil solutions were analyzed for P by colorimetric method [36]. Soil solution concentration was measured for planted and unplanted pots immediately at the time of each harvest.

To determine solid phase (extractable) P, a 10 g soil subsample from each pot was air dried then extracted with calcium acetate lactate (CAL) [37]. Phosphorous concentration in the extracts was determined using the previously mentioned method [36].

2.2.3. Root Length, Root Radius, and Specific Root Density Measurements

The roots were carefully collected by washing off the soil in a sieve with a 0.2 mm wide mesh. Roots were cleaned of any foreign materials and then spread on paper towels. The surface moisture on the roots was removed manually by applying uniform pressure using paper towels and finally the root fresh weight (RFW) was recorded. Afterwards, a representative fresh root material of different parts of the root system in each pot (upper, middle and apical) was cut in small pieces (5-10 mm). After fine cutting these root portions (1-3 mm), two sub-samples were taken accurately for the root length measurement, using the line intersection method [38]. Each fine-cut root sub-sample was dispersed in a known volume of water and an accurately measured volume of aliquot of the root soak was taken and poured in a plastic dish with a grid bottom with lines 12.5 mm apart. The total number of root intercepts with the vertical and horizontal grid lines was counted by means of a hand tally counter. The root length in the aliquot of the sub-sample was calculated using the following equation:

\[
RL = (11/14) \times GD \times N.
\]

Where, RL = Root length of the sample in the plastic dish in mm, GD = Grid dimension (12.5 mm grid squares), N = Number of intercepts. The root length in the fresh weight subsample was calculated from a volumetric relation between the aliquot and the subsample. The total root length of the plants was obtained from the weight relation between the subsample and the total weight. Assuming that the specific weight of roots is 0.1g mm\(^{-3}\), the mean root radius \(r_0\) was calculated as:

\[
r_0 = \sqrt[3]{\text{Root fresh weight (RFW)}} / \pi \text{ Root length (RL)}}.
\]

The specific root density or root length density \(RL_s\) was calculated by dividing root length (RL) by the root fresh weight and interpreted as mm root/g root.

2.2.4. Shoot and Root Growth Rates

This ratio relates the difference in shoot or root growth between the two harvests divided by the number of days between the two harvests: Shoot growth rate \((GR_s) = \ln(SW_2 - SW_1) / (t_2 - t_1)\). This equation also apply for root growth rate. Where, \(SW_1\) and \(SW_2\) are shoot dry weight at the first and the second harvests respectively, and \(t_1\) and \(t_2\) are number of days of the plants at the first and the second harvests respectively.

2.2.5. Shoot Demand (SD): Shoot Growth Rate in Relation to Average Root Length

This ratio relates the P acquisition load imposed by shoot growth to each root segment. It was calculated by dividing the shoot growth rate \((GR_s)\) by the average root length \((aRL)\) assuming exponential root growth. Shoot growth rate/root length \((GRs/RL) = ((SW_2-SW_1)/(t_2-t_1)) \times \ln((RL_2/RL_1)/(RL_2-RL_1)).\) Where \(RL\) is the root length [mm] and \(SW\) is the shoot dry weight [g] at two harvest dates \((t_2-t_1)\).

2.2.6. Net P Influx

The influx is the net amount of a nutrient that is taken up per unit root length (or root surface area) per unit time. Since direct measurement of the influx is not possible, only an average influx can be calculated for a given time period. At least two harvests are needed in which the nutrient content and root length of the plants are known. Assuming that the roots of young plants show exponential growth, the average influx was calculated [39]:

\[
In = \left\{ \frac{U_2 - U_1}{\ln(RL_2/RL_1)} \right\} / \left\{ \frac{(t_2-t_1)}{(RL_2-RL_1)} \right\}.
\]

Where \(In\) is the influx, \(U\) is the shoot P content [mol] at two harvest dates \((t_2-t_1)\) related to the root length between the two harvests \((RL_2-RL_1)\).

2.2.7. Calculating Efficiency Indicators

Different measures of P efficiency were determined at different P levels. P accumulated (mg P/pot) in shoot was calculated from the multiplication of shoot weight in g with tissue [P] concentration multiplied by 100. Phosphorous concentration [mg P/g dry matter (DM)] in the plant was obtained from dividing the total mg P accumulated per pot by the total dry mater of the plant per pot (g) divided by 10. Phosphorous uptake efficiency was calculated by dividing total P accumulated per pot (g/pot) by soil solution P supply amount or CAL-P supply amount (g/pot). Phosphorous efficiency ratios (PER) was calculated as shoot dry mass (g/pot) divided by total P accumulation (g/pot) [40]. Phosphorous utilization index (PUI) [41] was calculated by dividing seed yield (g/pot) by P content in whole plant [g P (g DM)\(^{-1}\)].

2.3. Statistical Analysis

All statistical analyses were carried out using SAS (SA Institute Inc., Cary, USA, Release 8.02, 2001). Comparisons of means between different treatments were carried out using the GLM procedure considering a fully randomized design. With multiple t-test, the Bonferroni procedure was employed in order to maintain an experiment-wise α of 5%.
3. Results

3.1. Effect of P Supply on Growth and Morphology

P deficiency affected the three cultivars differently in terms of root, shoot and total fresh weight (Table 1). Root weight of Anbar didn’t change significantly in different P regimes, while Kahhatat roots were significantly reduced in both low and high P levels as compared to medium P supply. In the other hand Noursi increased their roots under suboptimal P supply. Both traditional wheat cultivars had more root fresh weight than that of Anbar at all P supplies and the difference at suboptimal levels was more pronounced significantly. Shoot and total fresh weight of Anbar and Kahhatat were reduced at both low and high P levels, but Noursi maintained significantly similar shoot and total fresh weight under low, medium, and high P supplies. P deficiency at 0 added P/ pot caused chlorosis, necrosis and marginal scorching of old leaves, which led to shedding of some of the old leaves of the three cultivars under study. Toxicity symptoms appeared at 1g P/ pot in the three cultivars under study resulted in interveinal chlorosis and marginal necrosis, leading to shedding of old leaves and leaf desiccation, these toxicity symptoms were more pronounced in Anbar and the least in Noursi, while Kahhatat was intermittently affected.

Shoot dry weight of Anbar and Kahhatat was significantly reduced under suboptimal as well as under excess P supplies, while Noursi tuned its shoot mass to be not affected significantly under neither deficiency nor excess of P supply (Figure 1). Anbar and Kahhatat had similar shoot dry weight at P deficient supply and significantly less dry matter than Noursi, while the three cultivars didn’t differ from each other significantly at optimal and high P supplies.

Table 1. Effect of P supply on fresh weight (g/ pot) of wheat (Anbar, Kahhatat, and Noursi) plant parts (root, shoot, and biomass). For a given wheat cultivar and different P supply, means within each column followed by the same capital letter are not significantly different, means of the same P supply within cultivars followed by the same small letter are not significantly different. P< 0.05, n=4

| P supply (g/ pot) | Root         | Shoot        | Total        |
|-------------------|--------------|--------------|--------------|
|                   | Anbar        |              |              |
| 0                 | 2.87±0.62 A, b | 11.60±0.30 C, b | 14.88±0.52 B, b |
| 0.2               | 3.87±1.14 A, b | 19.14±2.50 A, a | 23.98±3.79 A, b |
| 0.5               | 2.17±0.45 A, b | 16.72±2.09 BA, a | 19.66±1.91 BA, b |
| 1.0               | 2.08±0.53 A, b | 12.45±0.55 BC, b | 15.12±1.24 BC, b |
|                   | Kahhatat     |              |              |
| 0                 | 7.26±2.12 B, ba | 14.36±1.00 B, ba | 22.39±3.05 B, ba |
| 0.2               | 10.51±1.84 A, a | 22.08±5.56 A, a | 35.05±7.25 A, a |
| 0.5               | 12.08±6.48 A, a | 22.36±3.27 A, a | 35.97±9.56 A, a |
| 1.0               | 5.94±1.61 B, a | 17.03±1.61 A, a | 24.26±2.38 B, a |
|                   | Noursi       |              |              |
| 0                 | 10.43±2.37 A, a | 18.26±3.32 A, a | 30.00±6.11 A, a |
| 0.2               | 6.48±2.95 B, ba | 18.23±0.79 A, a | 26.25±4.39 A, a |
| 0.5               | 8.88±3.25 BA, a | 21.09±2.60 A, a | 32.18±6.70 A, a |
| 1.0               | 4.97±1.14 B, ba | 15.76±1.56 A, ba | 22.08±2.20 A, a |

Figure 1. Effect of P supply on shoot dry weight (g/ pot) of wheat (Anbar, Kahhatat, and Noursi). For a given wheat cultivar and different P supply, means followed by the same capital letter are not significantly different, means of the same P supply within cultivars followed by the same small letter are not significantly different. P< 0.05, n=4
Root length (cm pot$^{-1}$) was reduced under low and high P supplies in Anbar and Kahhatat, while Noursi improved root length under P deficiency as compared to optimal P supply (Figure 2). Both traditional wheat cultivars (Kahhatat and Noursi) had higher root length than that of the introduced cultivar (Anbar) at all P supplies.

Root radius (µm) was statistically similar in different P levels in each wheat cultivars under study (Figure 3 A). Anbar had significantly thinner roots as compared to those of Kahhatat or Noursi at all respective P supplies. Both Kahhatat and Noursi had similar root radius at all respective P levels. Specific root density (SRD, cm root/ g soil) of all cultivars under study didn’t differ significantly in different P supplies (Figure 3B). Anbar had higher SRD than that of Kahhatat or Noursi at all respective P levels, and this effect was significant at low and medium P supplies.

![Figure 2](image2.png)

**Figure 2.** Effect of P supply on root length (cm/ pot) of wheat (Anbar, Kahhatat, and Noursi). For a given wheat cultivar and different P supply, means followed by the same capital letter are not significantly different, means of the same P supply within cultivars followed by the same small letter are not significantly different. P< 0.05, n=4

![Figure 3](image3.png)

**Figure 3.** Effect of P supply on root radius (A, µm) and specific root density (B, cm root/ g root) of wheat (Anbar, Kahhatat, and Noursi). For a given wheat cultivar and different P supply, means followed by the same capital letter are not significantly different, means of the same P supply within cultivars followed by the same small letter are not significantly different. P< 0.05, n=4
There was no clear trend among treatments concerning relative growth rate of roots (Figure 4 A). Relative root growth rate of Anbar (RRGR, cm root/ S) was similar at 0, 0.2 and 1 g P/ pot, while it was significantly reduced at 0.5 g P/ pot. In Kahhatat, this figure was reduced at 0g P/ pot only, while in Noursi this trait was similar at 0, 0.2 and 1 g P pot\(^{-1}\) but was significantly higher at 0.5 g/ pot. The three cultivars had similar RRGR at 0 g P/ pot and 1g P/ pot respectively. At 0.2 g P/ pot, Kahhatat had significantly higher RRGR than Anbar or Noursi, while at 0.5 g P pot\(^{-1}\), Anbar was significantly reduced as compared to Kahhatat or Noursi.
All cultivars tuned their shoot growth rates (RSGR, g shoot/ S) to less values as P supplies decrease but this trend was significant in Anbar only. The growth rate of Anbar shoot was significantly higher than those of both Kahhatat and Noursi when they grown under suboptimal P supply as well as at excess P supply, while at medium P levels, all cultivars had similar RSGR. Both traditional wheat landraces had similar growth rate of shoot at all respective P supplies.

Root shoot ratio (RSR, cm root g/ shoot) in all cultivars under investigation increased when P supply was deficient (Figure 5). RSR was significantly similar for all studied cultivars under low and high P supplies. Under optimal P level (0.2 g P/ pot), Kahhatat had significantly higher RSR than those of Anbar or Noursi and the later ones had similar values.

Shoot demand ((g shoot/ day)/ cm root) increased significantly as P supply increased in all studied wheat cultivars (Figure 6). Shoot demand on P in Anbar was significantly higher than that of both Kahhatat and Noursi under deficient and excess P supplies. But under optimal P supply (0.2g/ P pot), shoot demand was significantly the highest in Noursi and the lowest in Kahhatat and intermediate in Anbar.

**Figure 6.** Effect of P supply on shoot demand [RSGR, (g shoot/ day)/ (cm/ root)] of wheat (Anbar, Kahhatat, and Noursi). For a given wheat cultivar and different P supply, means followed by the same capital letter are not significantly different, means of the same P supply within cultivars followed by the same small letter are not significantly different. P< 0.05, n=4

**Figure 7.** Effect of P supply on P content (A, mg/ 100mg DM) and accumulation (B, mg/ pot) of wheat (Anbar, Kahhatat, and Noursi) shoot. For a given wheat cultivar and different P supply, means followed by the same capital letter are not significantly different, means of the same P supply within cultivars followed by the same small letter are not significantly different. P< 0.05, n=4
3.2. P Concentration

The three studied cultivars reacted significantly with increasing P supply in terms of P content (mg P/100mg DM) and accumulation (mg P/pot) (Figure 7). Anbar P content reflects the increase in each P supply significantly in more sensitive way as compared to the traditional wheat cultivars. Comparing the three cultivars at respective P supplies, Figure 7A reveals the similarity of the cultivars at 0.5 and 1 g P/pot, while at 0 g P/pot, Anbar had the same P concentration as Kahhatat as and significantly less than Noursi at 0.2 g P pot\(^{-1}\). Anbar had significantly less P content than Noursi, while Kahhatat was intermediate. Additional, only at very high P supply, Anbar accumulated less P than Kahhatat and Noursi.

3.3. Phosphorous Use Efficiency (PUE)

3.3.1. Soil Parameters

Available phosphorous in soil solution (mg P/L soil solution) as well as calcium acetate lactate (CAL) extractable (mg P/100g soil) P measured in the rhizosphere of all cultivars under study reflected significantly the increase of external P supplies (Figure 8). At all respective P fertilization levels, the three wheat cultivars depleted similar soil solution P and CAL-P. Buffer capacity of P of soil hosting the three cultivars was the highest when P supply was deficient, then decreased significantly at medium and excess P supplies. P buffer capacity of soil cultivating all cultivars under study had similar values at all respective P levels.

![Figure 8](image_url)
3.3.2. P uptake Rate (Influx)

Phosphorous uptake rate (μmol P/ cm root/ S) increased significantly with increasing P supply in the three cultivars under study (Figure 9). The introduced wheat cultivar (Anbar) responded continuously to increasing P supply by improving the P influx in their root cells, while the traditional wheat cultivars had the saturation point at 0.5 g P/ pot. All cultivars under study showed similar values of P influx at 0, 0.2, and 0.5 P/ pot, while at 1g/ pot, Anbar and Noursi were significantly similar but Kahhatat had reduced P uptake rate significantly.

Figure 9. Effect of P supply on P uptake rate (μmol P/ cm root/ S) of wheat (Anbar, Kahhatat, and Noursi). For a given wheat cultivar and different P supply, means followed by the same capital letter are not significantly different, means of the same P supply within cultivars followed by the same small letter are not significantly different. P< 0.05, n=4

Figure 10. Effect of P supply on percentage recovery of P (soil solution, CAL extractable) of wheat (Anbar, Kahhatat, and Noursi) shoot. For a given wheat cultivar and different P supply, means followed by the same capital letter are not significantly different, means of the same P supply within cultivars followed by the same small letter are not significantly different. P< 0.05, n=4
3.3.3. P Recovery

Recovery of phosphorous by the three studied cultivars at different P supplies was interpreted by relating P accumulation in shoot to total soil solution P/ pot (Figure 10A) and total CAL-P/ pot (Figure 10B). All cultivars under study recovered decreasing fractions from the soil solution supplied by increasing P levels significantly. At zero g added P supply/ pot, the cultivars under study depleted 12.4 times (Anbar), 13.3 times (Kahhatat), and 15.6 times (Noursi) of the nutrient solution that can the soil potentially provide. At optimal P supply (0.2 g added P/ pot), the studied cultivars depleted 3-4 times of the nutrient solution provided from soil at this P level. While at 0.5 and 1g added P/ pot, the cultivars under investigation depleted 1.3-1.8 times and 0.35-0.57 times of the nutrient solution provided by soil fertilized with 0.5 and 1g added P/ pot respectively. The three cultivars didn’t differ significantly among each other in depleting nutrient solution at each respective P supply.

In terms of the recovered partitions of the CAL extractable P, Figure 10B reveals that at 0 added P supply, Anbar and Kahhatat similarly depleted nearly 10 percent of the total extractable P/ pot, while Noursi depleted significantly higher value (nearly 16%). At 0.2g added P, Kahhatat depleted less percentage (12.6%) than Anbar (15.8%) while this figure for Noursi was similar to those of both Anbar and Kahhatat. At 0.5g added P supplies and 1g added P/ pot, the cultivars under study used similar partitions at each respective P supplies; 14.8-17.2% and 6.8-7.9% at 0.5 and 1g P supplies respectively. Generally, the partitions recovered from CAL extractable P has a trend to increase from 0 to 0.5g P and significantly decreased at 1g P per pot in all cultivars.

3.3.4. Phosphorous Utilization Efficiency

Phosphorous use efficiency (Figure 11) interpreted as phosphorous efficiency ratio (PER) and phosphorous utilization index (PUI), decreased significantly in dramatic way in the three wheat cultivars as P supply increased. All cultivars had statistically similar values for each efficiency indicator separately at each respective phosphorous supply, but there is a trend that Anbar is better P utilizer than Kahhatat and Noursi at all P supplies except at 0 added P.

Figure 11. Effect of P supply on PER (A, g shoot/ g P in shoot) and PUI (B, g shoot/ %P in shoot) for of wheat (Anbar, Kahhatat, and Noursi) shoot. For a given wheat cultivar and different P supply, means followed by the same capital letter are not significantly different, means of the same P supply within cultivars followed by the same small letter are not significantly different. P< 0.05, n=4
4. Discussion

4.1. Growth and Morphology

Biomass production can be a reliable parameter for screening efficient cultivars [42], thus it is used as an important plant trait in growth analysis [43], and an indicator to economic yield [44]. P deficiency affects plants by reducing leaf expansion, auxiliary bud and shoot canopy growth, reduces the plant's photosynthetic surface area and carbohydrate utilization [6]. P deficiency negatively affects vegetative growth, limits the formation of reproductive organs, results in premature leaf senescence, delays flower initiation [45], decreases number of flowers [11], restricts seed formation [46], and finally contributes to growth and yield reductions.

In agreement with our results concerning fresh (Table 1) and dry weight (Figure 1) production, P nutrition had a positive influence on fresh and dry matter production of Anbar and Kahhatat as reported in sunflower [22], safflower [45], and wheat [10,47]. Noursi was less sensitive to P deficiency than Anbar and Kahhatat in terms of biomass accumulation.

Longest roots were observed when the cultivars under study were grown at optimal P supply. The introduced wheat cultivar (Anbar) wasn’t able to enhance root length under P deficiency as both traditional wheat cultivars did (Kahhatat and Noursi). The most efficient cultivar that maintained higher root length under P deficiency was Noursi and the lowest one was Anbar, while Kahhatat was intermediate. All cultivars under study reduced their root growth under high P supply (Table 1 and Figure 2). This indicate that, traditional wheat cultivars under study can increase the root size (weight and length) under low P supply which enable the plant to overcome low P availability by exploring more soil volume [28]. Efficient plants can also modify their root system including fineness [48] and density [49] for greater absorbing surface under low P supply, a response was not observed in wheat cultivars under study neither for root radius (Figure 3A) nor for root density (Figure 3B). Anbar had finer roots significantly than both traditional wheat cultivars and also the former has larger specific root density than the laters at all P levels, giving Anbar efficiency genetic traits over both traditional wheat cultivars. Nutrient efficiency of plant species under suboptimal nutrient supply is also determined by slow shoot growth rates [21], hence, a plant species with a low shoot growth rate such as both traditional wheat cultivars under study could be considered more efficient than the introduced one under suboptimal P supply (Figure 4B). In the other hand, plants that maintained high root growth rate under P deficiency, are more efficient in overcoming P deficiency by exploring more soil, a response was not clearly observed in the cultivars under study (Figure 4A).

The root shoot ratio (Figure 5), interpreted in this study as the ratio between root length (cm) and shoot weight (g) is a basic parameter of nutrient acquisition by plants [50]. The three studied cultivars increased their root length-shoot ratio under P deficiency [51]. Both traditional cultivars had significantly more roots per each g of shoots under low P as well as high P supplies. This increase in root-shoot ratio (RSR) under low-P supply -observed in the three cultivars and was more pronounced in traditional cultivars as compared to the introduced one- have been regarded as a kind of adaptive response of roots to low external P levels [50]. An increase in RSR in P deficient plants is due to the more reduction of shoot growth than root growth [52], and can be attributed to higher export rates of photosynthates to the roots to increase root surface area for P-absorption enabling the stressed plants to acquire more P from the surrounding environment [53]. Therefore, P-deficiency induce more dry matter partitioning in favor of heterotrophic tissue by reducing growth of photosynthetic tissue and thus allocate more biomass to roots when P is limiting for their growth [50]. In contrary with our results, many Lupinus species show marginal biomass partitioning to roots as dependent on P supply [54], although some of them were indicated as P-efficient.

As mentioned above, roots have mainly to meet the nutrient demand exerted by shoot growth. Hence, the shoot growth rate together with the required P concentration in the shoot is a measure of the demand, the shoot is putting on each root segment [21]. Therefore, shoot demand (SD) on the root is interpreted as the P acquisition load imposed by shoot growth on each cm of root and is calculated by dividing the shoot growth rate by the average root length (RL). Figure 6 shows the shoot demand on roots of wheat cultivars under study as affected by increasing P supplies. SD of Anbar was higher as compared to both Kahhatat and Noursi at low and high P supplies. This high SD of the introduced cultivar was attributed to both higher values of shoot growth rate (Figure 4B) and lower root length/ shoot dry matter ratio in Anbar as compared to both Kahhatat and Noursi (Figure 5).

4.2. P Concentration and Accumulation

Crop species that can grow normally with low tissue P concentrations due to efficient use of P was reported to be more tolerant to low P conditions than that exhibit high P concentrations in the tissues [22,53]. These plants can maintains relatively low tissue concentration of P due to efficient incorporation of the external P into residue-P [55], or because the vacuole acts as a P reservoir to maintain a constant cytoplasmic P concentration [56]. Anbar maintained lower P concentration than Noursi at all P supplies and the deference was statistically significant at 0 and 0.2 P supplies (Figure 7). Kahhatat tissue-P concentration was intermediate at all P levels but was significantly similar to Anbar and deferent from Noursi at the most deficient P supply.

4.3. P Uptake Efficiency

4.3.1. Soil Parameters

Differences in P efficiency among genotypes can be studied in field or in pot experiments with soil or with nutrient solution. However, contradictory results may be obtained when plants nutrient efficiency is evaluated using these three growth media. Results from field trials cannot be easily repeatable due to soil heterogeneity and complexity [57]. However, pot trials compared to field
trials have the advantage that uniform growth conditions can be set regarding fertilization and soil homogeneity and also climatic conditions can be controlled. On the other hand, although nutrient solution experiments can be easily repeated, they can cover only part of the factors, that can contribute in genotypic differences in nutrient efficiency by plants growing in soil. For instance, the root growth conditions and P uptake are largely different between nutrient solution experiments and soil. Therefore, the relevance of plant and non plant factors (soil) in P uptake would be different according to the experimental methodology used [31,58]. Plant species and even varieties of the same species differ in their ability to grow in soil low in nutrients [22,59]. An efficient plant can utilize mobile, available, and fixed nutrients in soil and can exploit more soil in order to maintain required rate of nutrient uptake by roots [59]. As discussed earlier, plant properties affecting uptake of nutrients from soil include the size of root system, morphological root properties and kinetics of ion absorption by roots [30]. Other properties are related to soil in which the supply of mineral nutrients to plants is the result of interactions between the nutrient availability in soil and the ability of plants to absorb this nutrient. Both soil and plant properties are therefore, control the nutrition of plants. Movement of nutrients from soil to root is brought about by mass flow and diffusion [30,60]. Mass flow is the convective transport of nutrients dissolved in the soil solution moving to plant roots as a result of shoot transpiration, while diffusion is the movement of a nutrient from one region to adjacent regions where particular nutrient has lower concentration [30]. The diffusion of phosphorus “flux” through the soil to the plant’s roots, is –in many soils- the mechanism governing 90 to 98% of the P supply to the roots [50,61], while the rest is provided by mass flow [60]. Because a concentration gradient is required for diffusion to occur, the plant root takes up nutrients, lowers the nutrient concentration on its surface, and thus creates a gradient unless mass flow counteracts the process. Therefore the decrease of the nutrient concentration at the root surface is determined by the uptake properties of roots. The three cultivars under study, had similar soil solution P and CAL-P at all respective P levels (Figure 8) which indicate that investigated cultivars have similar ability to deplete available P at respective P supplies.

4.3.2. P uptake Rate (Influx)

Plant roots act as a sink for soil nutrients, and it is the plant that initiates nutrient transport from soil to root by depleting P ions at the surface of the root cell (influx). P influx by roots lower the initial ion concentration of the soil solution around roots, create a concentration gradient from soil toward the root, cause diffusive flux, and disturb the equilibrium between P ions on the solid phase with those in the liquid phase, cause their release from soil particles into solution [30]. Therefore the extension of the depleted zone and the degree of depletion is basically the result of interactions between plant and soil parameters. Superior species may have higher uptake rates per unit root and time [29], and increase diffusion towards roots by steeping the concentration gradient [62]. Phosphorous uptake rate (influx, µmol P/ cm root/ S) was statistically similar in the three cultivars under study at all respective P supplies, except at very high P level where Kahhatat was inferior as compared to the others. This influx increase significantly with increasing P levels in all studied cultivars. In contrary with our findings (Figure 9), other researchers reported an increase in P uptake rate under P deficient supply [63]. In our research, the P influx in the roots of the three cultivars was reduced under low P supply which indicate that the three cultivars don’t use this mechanism to enhance P uptake under P deficiency. It was speculated that the efficiency of the uptake system is of minor importance for P acquisition from soils because transport of P to the root surface rather than the uptake is the limiting step [27]. Therefore it is less likely that selection for efficient P uptake kinetics will contribute to more efficient P acquisition from low-P soils, and accordingly, choosing this trait will be not applicable as a selection criteria for P uptake efficiency in wheat cultivars under study.

4.3.3. P Recovery

P recovery was interpreted as the relation between accumulated P in DM and supplied P (soil solution P or CAL extraction P). The P supply represented as soil solution used in this investigation tested in pots before planting was 0.15, 3.24, 13.54 and 38.51 mg P/ L soil solution after adding respective P levels (0, 0.2, 0.5, and 1 g P/ pot). All studied cultivars depleted similar repeated times of soil solution P at all P supplies (Figure 10A), Anbar and Kahhatat depleted less times of solution P than Noursi at low P supply but the difference was not significant. The normal concentration of P in soil solution in the field was reported in the order of 0.32 – 19.37 µmol P/L [64], and this concentration can be depleted rapidly by growing roots in soil [65]. Other researchers reported that, P is present in the soil solution at a concentration less than 0.06 mg/ L and in agreement with our results this concentration is depleted many times during the life span of the growing plants [66]. However, in P-limited soils, the quantity of labile P may be insufficient to maintain P solution concentration against depletion by plant root. It has been reported that the P concentration in soil solution (external P requirement) necessary to achieve maximum growth differs widely among crops [64,67]. Hence, at a low P concentration in soil solution, P efficient plants may be either those with a low external P requirement or those which are able to achieve their external requirement by developing morphological and/or physiological root mechanisms.

The extractable P content (mg P/ Kg soil) of the soil after adding external P in this study were 8.66, 20.30, 74.74 and 133.33 in respective P supplies (0, 0.2, 0.5, and 1.0g P pot^{-1}). Both species also depleted similar fraction of extractable P at all P levels except at low P level where Noursi recovered significantly higher than both Anbar and Kahhatat indicating the efficiency of Noursi in solubilizing P from unavailable P pool in the soil as compared to Anbar and Kahhatat (Figure 10B). Besides the relationship between P concentration and growth of plants, extractable P in the soil can be a measure of its availability. Although, ions not readily released from the soil matrix could be of minor importance to plants, but there is some evidence that these phosphate fractions may play a role in supplying P to plants [68].
4.4. P Utilization Efficiency

Efficiency ratio (ER), defined as the biomass production per unit nutrient accumulated, is widely used for the comparisons of efficiency under moderate to severe nutrient deficiency stress [22,40]. It is a valuable parameter in differentiating plants into efficient and inefficient utilizers of the absorbed nutrients [22,46], and has been used extensively to describe internal nutrient requirements in many agronomic species [18,20,22,46,69]. The utilization index (UI) is defined as biomass produced per unit of tissue nutrient concentration [18,41,69], that unlike the efficiency ratio, UI takes differences in the amount of produced biomass into consideration. UI was proposed to avoid the interpretation of the dilution effect under low nutrient supply as utilization efficiency when interpreted in terms of ER [18,22,69]. The continuously increasing values of P ER and P UI to produce yield (DM), exhibited by wheat cultivars under study (Figure 11), in response to decreasing nutrient supply, represents the general response of the adaptation of different cultivars to nutrient-poor environments by enhancing their nutrient-use efficiency [67]. Similar observations have been found for safflower as compared to sunflower [22], and among wheat cultivars [10]. However, the ability of the three wheat cultivars under investigation to utilize P similarly at respective P supplies in terms of P ER and P UI indicate that they have similar utilization efficiency in terms of these parameters at all studied P supplies.

5. Conclusion

New alternative crops need to be developed, that can acquire and use soil P more efficiently in focusing on cultivars which represent nutrient efficiency traits. Plant cultivars vary in their P use efficiency at different P supplies by using different strategies related to uptake efficiency that could be used in selecting or breeding programs. P uptake efficiency depends on those factors related to plant parameters and those related to soil parameters. P efficient crop may increases root size, root length, specific root density, root-shoot ratio, nutrient influx and reduces root diameter, shoot growth rate and shoot demand on roots. The ability of the crop species to increase P solubility in the rhizosphere (P intensity and capacity) and depleting more soil solution and extractable P are considered as mechanisms of P uptake efficiency in terms of soil parameters.

Traditional and modern cultivars under study had similar overall uptake (P influx and recovery) and utilization (ER and UI, P status in DM) efficiency. In terms of specific efficiency parameters, traditional cultivars had the superiority in having more root size, higher root shoot ratio, slower shoot growth rate and less shoot demand on roots for P, but were inferior in having thicker roots and less specific root density. Investigating P use efficiency mechanisms could be a useful tool in selection programs to separate plant cultivars to superior and inferior, but using different measures of utilization efficiency parameters could be in some cases misleading.

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Competing Interests

None declared.

Abbreviations

P- Phosphorus, CAL- Calcium acetate lactate, mm- millimeter, L- Liter, ICP- Inductively coupled plasma, RFW- Root fresh weight, RL- Root length, GD- Grid diameter, r0- root radius, GRs- shoot growth rate, GGr- root growth rate, SW- Shoot weight, SD- shoot demand, t- time, In- influx, U- Shoot P content, DM- Dry matter, PER- Phosphorous efficiency ratio, PUE- Phosphorous use efficiency, PUI- Phosphorous, SRD- specific root density, RRER- Relative root growth rate, RSR- root shoot ratio, ER- Efficiency ratio, UI- Utilization index.

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