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EVALUATION THE EXPRESSION PATTERN OF *TAPHT1* AND *TAPHO2* GENES IN WHEAT (*TRITICUM AESTIVUM* L.) UNDER PHOSPHORUS DEPRIVATION CONDITION

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Abstract

Phosphorus (P) is an important macronutrient with critical functions in plants. Low phosphorus availability is a major abiotic factor constraining wheat growth. Phosphate (Pi) transporters, which mediate Pi acquisition and Pi translocation within the plant, are key factors in Pi deficiency responses. However, their relevance for adaptation to Pi limitation, particularly in wheat, is still unclear. A hydroponically study was conducted to evaluate the expression pattern of *TaPht1*and *TaPHO2* genes in 8 wheat genotypes (*Triticum aestivum* L.) in response to three P application rates. Seedlings were hydroponically grown in the adequate 0.1*mM*, deficit 0.02*mM* and deprivation (0) P. A factorial experiment was arranged in Randomized Complete Block Design (RCBD) with three replicates. Dry weight of shoot and root, P contents in shoots and roots were measured. The expression of *TaPht1*and *TaPHO2* genes with housekeeping *TaQhnRNP* gene was evaluated. The measured criteria (shoot and root dry weight and shoot and root P content) were significantly different between P levels conditions. Latifya and AbuGhraib grown in adequate P (0.1*mM*) and deprivation (0P) medium produced higher shoot dry weight (0.446 and 0.385 g) and (0.118 and 0.124g), respectively. Latifya produced higher root dry weight (0.119 and0.580 g) at 0.1 and 0.02*mM* P, respectively, Babylon (0.66g) at 0 P level. The genotype AbuGhraib had higher content of P in shoot and roots at 0.02*mM* P which gave 0.560 and 0.390%. The expression of *TaPHT1* and *TaPHO2* genes were increased after exposed to low-P level and deprivation, overexpression was in AL-Fatah 36.3 folds and 9.5 folds of gene *TaPHT1* at 0.02*mM* and 57.9folds of *TaPHO2* gene at P deprivation, Latifya 119 folds of *TaPHO2* gene at 0.02*mM*P. This study showed a large variation in shoot and root dry weight, shoots and root P content and gene expression among wheat genotypes under various P concentrations. The findings of this study reveal that AbuGhraib andLatifya are efficient and responsive genotypes, which have potential for better growth in P limited environments. However, these results should be confirmed under field conditions.

Key words: Wheat, P deprivation, Pi acquisition, gene expression.

Introduction

Phosphorus (P) is the second most limiting nutrient in plants after nitrogen, being involved in numerous cellular processes such as protein activation, energy transfer, signaling andregulation of carbon metabolism (Xu *et al.*, 2018). P. deficiency is a generally widespread stressor occurring in natural and agricultural environments. Consequently, global agricultural crop production has been severely affected (Heuer *et al.*, 2017). Plants acclimate to Pi deficiency by modifications of growth parameters and metabolism or genes expression and protein production. The genes such as *PHR1-IPS1-miR399-UBC24/PHO2-PHT1/PHO1, TaPHO1-10, aPht1*-10 and molecular mechanisms involved in Pi stress response are specifically induced during Pi deficiency and not under any other modes of stress known to alter Pi homeostasis (Kisko *et al.*, 2018).

The wheat genome contains several *TaPHT* members that could be divided into four subfamilies, *PHT1* (*TaPHT1.1–1.13*), *PHT2* (*TaPHT2.1*), *PHT3* (*TaPHT3.1–3.3*) and*PHT4* (*TaPHT4.1–4.6*). Their transcripts demonstrate enhanced expression in Pi limited roots and shoots (Shukla *et al.*, 2016). Under Pi deprivation, Pi uptake increases and involves a high-affinity *PHT1* member *TaPT2* (Guo *et al.*, 2014). Down-regulation of *TaPHT2.1* was able to induce a pronounced decrease in Pi accumulation in both sufficient and Pi-deficient wheat (Guo *et al.*, 2013). Under P deficient

conditions, *PHT*s show activity in shoots and roots, as P stress causes the gene to become up-regulated under P stress (Huang *et al*., 2011). At the molecular level the PTs (Phosphate transporters) in the cell membrane of the root epidermal cells and root hairs facilitate the acquisition of P; few such Pts have been characterised in wheat (Liu *et al*., 2013; Guo *et al*., 2014). Root expressed *Pht1* transporters, predominantly induced in root tips and root hairs (Glassop *et al*., 2005), have been identified in a broad range of different plant species including wheat, indicating their involvement in initial root Pi acquisition from the soil solution (Wang *et al*., 2013).

The *Pht1* genes are involved in P uptake against a sharp concentration gradient, as the root cells may contain 10,000-fold higher soluble P concentration than the soil solution (Yan *et al*., 2004). Ouyang *et al.,* (2016) identified three *TaPHO2* genes in wheat that are involved in P uptake and translocation of P. They found that a *TaPHO2* mutant had a higher total P concentration in the grain, higher biomass production and greater grain yield than the wild type under low P conditions. *TaPHO2* expression was found to be related to root and shoot growth, shoot Pi accumulation andactivity of some *PHT1* transporters (Ouyang *et al*., 2016). In this context, the Pi transporter gene family *PHT1* from wheat has been recently identified, consisting of 16 phylogenetically distinct transporters (Grun *et al*., 2018). Some *PHT1* proteins are abundant in the root epidermis, facilitating the uptake of Pi that is released from organic matter and soil particles against a steep concentration gradient (Nussaume *et al*., 2011). Gene modification is a potential mean of enhancing Pi starvation tolerance (Wang *et al*., 2013). *Pht1* transporters, which are preferentially or exclusively expressed in roots, were found in wheat (Teng *et al*., 2013; Wang *et al*., 2013). Exploring the molecular mechanisms in regulation of P uptake and utilization may help to breed wheat with improved PUE (Ouyang *et al*., 2016), they indicated that *TaPHO2s* involved in phosphorus uptake and translocation and molecular engineering *TaPHO2* shows potential in improving wheat yield with less phosphorus fertilizer.

Bread wheat (*Triticum aestivum* L.) is the leading widely grown food cereal around the globe, due to it wider adaptability as well as quality of nutritive values than other cereals. Similarly, in terms of production and area it is also stands first. It is as a strategic crop which has a significant role on the national economy of the third world countries (Yildirim *et al*., 2018). Whereas, it demand is increasing day by day to meet the food security of increasing population (Jahan *et al*., 2019). Therefore, improving P fertilization efficiency in wheat cropping is a major goal in order to achieve a more sustainable agricultural production andimportant to the sustainable use of P resources. The last can be achieved by improving the availability of P fertilizers in soil, such as by avoiding Pi sorption to soil particles and/ or by the development of P use - / acquisition - efficient plants (de Souza Campos *et al*., 2019). Phosphorus is known that crop genotypes can differ in the efficiency of acquisition and utilisation of nutrients. In semi-arid environments, the mobility of P is further reduced by water deficits in the soil restricting diffusion; this is a common problem on middle and south Iraq. Thus the objectives of this study was to evaluate gene expression levels of *TaPht1*, *TaPHO2* genes with reference gene *TaQhnRNP* as a response to limited Pi - availability and deprivation.

Materials and **Methods**

Plant Materials

Seeds of wheat genotypes (Ibaa95, Ibaa99, Uruk, AbuGhraib, Latifya, Tamooz2, Babylon and AL-Fatah) acquired from Ministry of Agriculture/ agricultural Research Service – Iraq. The selected seeds carry official certificates and they have adapted to the environmental conditions of Iraq for many years. They cultivated in the central and southern of Iraq. The ability of these genotypes to tolerate phosphorus deprivation has not yet been tested.

Growth Conditions and **Nutrient Solution**

Seeds were germinated in Petri dishes on sterile water-soaked paper tissue and irrigated with distilled water in hydroponic laboratory. The experiment was conducted in two different timetables continually, the Petri dishes were incubated at 72 hours darkness and covered with a black polyethylene sheet and then it turned on the light for 7 days for germination. Ten day-old, uniform and healthy seedlings were transferred to pots at 3 cm depth in an artificial soil (Paralit) in three replicates as a factorial experiment in a Completely Randomized Block Design (CRBD) and each pot contained 5 plants thining to one plant after 7 days in hydroponic system. They were tested under P deficient (0.02 *mM*), Deprivation(0) and adequate (0.10 *mM*)P levels. The temperature was maintained between 18 ^{o}C / 15°C during the day/night respectively. Humidity was controlled at 60-70 during the experiment days. Light times were 16/8 day/night respectively. The intensity of the light radiation was about 70000 lux or 1300 PPF (µmol m-2 s-1) (PPF, Photosynthetic photon flux). Nutrient solution was used in the experiment, which contains all the elements necessary for the growth of wheat plants perfectly. Wheat plant were supplied with nutrients solution containing macronutrients such as 15.7143 ml NH 4NO3, 27.8025g

Table 1: Primers pair for RT- PCR.

No.	Gene		Primer sequence	Reference
1	*TaPht1*	F	CGACACCATTGCTCCGACTG	Grun *et al.*, 2018
		R	TCAAACACACCAACCATGCACG	
2	*TaPHO2*	F	GGAGAAGAACTCCATCACGTACAACG	Ouyang *et al.*, 2016
		R	GGCAAGTGAAGTGCTCCTTGACGA	
3	*TaQhnRNP*	F	TTGAACTTGCCCGAAACATGCC	Grun *et al.*, 2018
		R	CACCTTCGCCAAGCTCAGAAC	

KNO3, 25.971 g $Ca(NO_3)_2$, 27.115 g $MgSO_4$ and 55 ml NaFe(III)EDTA. Whereas, micronutrients containing 650 µl $CuSO_4$, 13.75 ml $ZnSO_4$, 1.375 ml Na2MoO$_4$, 11 ml H_3BO_3, 11 ml $MnCl_2$. PH is between 6.5-7.0. Nutriet solution represent 10% from total solution. Plants were grown in hydroponic system for 45 days.

Plant sampling

After the period of 45 days of P treatments was complete, the seedlings were harvested, washed with distilled water and shoot and root parts were separated at the crown level immediately, subsequently, plants were then oven -dried at 70°C for 48 h (to a constant weight), then they were cooled in a dry environment and the shoot and root were weighed for dry weight by a sensitive balance.

Pi Acquisition

Plant samples were taken from different treatments and P content was estimated in shoot and root. Samples were washed with distilled water and HCl (0.01N), then sectioned and oven-dried at 70°C during 48 h till constant weight. They were ground and stored in glass containers until analysis. Each sample was separately analyzed for P concentration. Each sample was weighed (0.5g) and digested in 10 mL H_2SO_4-H_2O_2 for 24. After digestion, samples were filtered and the volume of each sample was adjusted to 50 ml using deionized water. Total P of samples was analyzed by atomic absorption (FAO, 2008).

Molecular Study

- Preparation of Root Samples:

Samples were collected in triplicate per treatment. Outer surface of the roots were washed with distilled water to remove residual contaminants. In addition, Scissors and forceps were washed with ethanol (70 v/v) and then with distilled water after each sample. After cutting of root samples, 1 ml of RNA secure solution (Bioland Scientific LLC, USA) was added to the ependrof tubes for each sample to protect RNA products from degradation until RNA extraction.

RNA Extraction

Ribonucleic acid (RNA) extraction is the first step to study gene expression. Total RNA was extracted from wheat root samples using TRIzol® protocol. The principle method of RNA extraction was related to using Traizol solution. Traizol is an acid guanidinium thiocyanate-phenol-chloroform mixture; It is a powerful method for DNA/ RNA extration (Chomczynski and Sacchi, 1987). This procedure was done using commercially available RNA extraction kit (GENEzol Tri RNA Pure Kit - Geneaid - Bioner, Korea) according to manufacturer's instruction.

Primers

Specific primers (*TaPht1* and *TaPHO2*) were used in this study, forward and reverse sequence of the primers and product size are presented in Table 1. These two primers pair genes with the reference gene *TaQhnRNP* as internal standard for normalization were used.

One Step RT-PCR

GoTaq® 1-Step RT-qPCR System is a reagent system for quantitative analysis of RNA using a one-step reverse transcription-quantitative PCR (RT-qPCR) protocol. Utilizing GoTaq® 1-Step RT-qPCR System combines the benefits of (i) GoScript™ Reverse Transcriptase. (ii) GoTaq® qPCR Master Mix for efficient, sensitive and linear one-step RT-qPCR quantification over a wide range of RNA template inputs. This kit contains a fluorescent DNA-binding dye named BRYT Green® dye that displays greater fluorescence enhancement upon binding to dsDNA. Table 2 lists PCR reaction components required for synthesis of cDNA from RNA and gene expression in one step, the therm cycler program was carried out following procedure (Table 3). Normalization of $\Delta\Delta C_T$ data was carried depending on the Livak and Schmittgen (2001) as follow:

Table 2: PCR reaction Components for synthesis of cDNA from RNA and gene expression in one step.

Master mix components	Stock	Unit	Final	Unit	Volume (µl)
qPCRMaster Mix	2	X	1	X	5
RT mix	50	x	1	x	0.25
MgCl2					0.25
Forward primer	10	µM	1	µM	0.5
Reverse primer	10	µM	1	µM	0.5
Nuclease Free Water					2.5
RNA		ng/µl		ng/µl	1
Total volume					10
Aliquot per single rxn	9µl of Master mix per tube and add 1µl of Template				

Table 3: Conditions of RT_PCR program.

Steps	°C	m : s	Cycle
RT. Enzyme Activation	37	15:00	1
Intitial Denaturation	95	05:00	
Denaturation	95	00:20	
Annealing	60	00:20 acquiring on Green	40
Extension	72	00:20	

Table 4: Effect of Pi treatments on shoot and root dry weight and content of P.

Pi treat-ments	Shoot dry weight (g)	Root dry weight (g)	Shoot content of P (%)	Root content of P (%)
0	0.103	0.036	0.32	0.265
0.02	0.139	0.05	0.404	0.343
0.1	0.285	0.051	0.515	0.415
LSD	0.0265 *	0.0134	0.0014 *	0.001 *

Folding $=2^{-\Delta\Delta CT}$

$\Delta\Delta CT = \Delta CT$ Treated - ΔCT Control

$\Delta CT =$ CT gene - CT House Keeping gene

Statistical Analysis

The data collected for various growth parameters were subjected to ANOVA using the Statistical Analysis System- SAS program (SAS, 2012) according to Randomized Completely Block Design-RCBD with three replicates and treatment means were differentiated by Least Significant Difference (LSD) method at $P\leq0.05$.

Results and Discussion

Shoot and root dry weight

The genotypes differ significantly in growth response at adequate (0.1*mM*), deficit (0.02*mM*) and deprivation (0) P levels. A significant ($P\leq0.05$) interactive effect was observed among genotypes and P level. Decrease P levels altered the shoot dry weight values significantly ($P\leq0.05$) when compared to control plants. The reduction was by 51.23 and 63.86%, or shoot and 29.41% for root (Table 4). The genotype, Ibaa99 had lower shoot dry weight

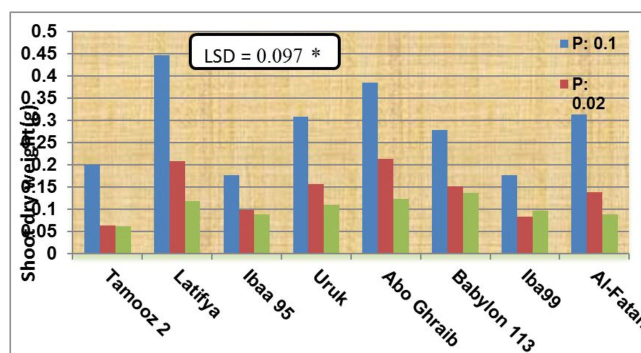

Fig. 1: Mean values for shoot height of genotypes under Pi treatments.

(0.119). Oppositely Latifya and AbuGhraib genotypes had higher shoot dry weight (0.257 and 0.24og), respectively. The maximum root dry weight values of 0.055 and 0.054g shown by genotypes AbuGhraiband and AL-Fatah, while minimum dry weight (0.026g) shown by Ibaa99 genotypes. Other genotypes had medium weight of shoot and root (Table 5).

All the wheat genotypes exhibited remarkable differences in their dry weight at deficient, deprivation and adequate P supplies in the growth medium. The genotypes Latifya and AbuGhraib grown in adequate P (0.1*mM*), low P availability (0.02*mM*) and deprivation (0P) medium produced higher shoot dry weight (0.446 and 0.385 g), (0.209, 0.213g)and (0.118and 0.124 g), respectively. Whereas the genotype Tamooz2 produced lower dry matter (0.176, 0.063 and 0.061g, respectively (Fig. 1). Root dry weight of genotypes differed significantly ($P\leq0.05$) when subjected to P levels in medium. The comparison of mean showed that Latifya and Uruk produced higher weight (0.119 and 0.089g), respectively at 0.1 *mM* P and Babylon and AL-Fatah (0.055 and 0.66g) at 0 P level. While the least performing genotypes AL-Fatah, Ibaa95 and Babylon at which produced of 0.024 each at 0.1 *mM* P and Uruk (0.022g) at 0 P level. The rest of the genotypes had values ranging between these mean. On low-P level (0.02*mM*), Ibaa99,

Table 5: Effect of genotypes on shoot and root dry weight and content of Pi.

Traits	Genotypes								
	Tamooz2	Latifya	Ibaa95	Uruk	Abo-Ghraib	Babylon	Ibaa99	Al-Fatah	LSD 0.05
Shoot D.W. (g)	0.107 ±0.07	0.257 ±0.19	0.139 ±0.06	0.191 ±0.09	0.240 ±0.13	0.189 ±0.09	0.119 ±0.05	0.179 ±0.11	0.0592 *
Root D.W. (g)	0.035 ±0.01	0.063 ±0.08	0.050 ±0.04	0.052 ±0.05	0.055 ±0.05	0.042 ±0.02	0.026 ±0.01	0.054 ±0.02	0.0345 *
Pi shoot content (%)	0.393 ±0.08	0.438 ±0.09	0.428 ±0.06	0.388 ±0.08	0.457 ±0.09	0.398 ±0.08	0.426 ±0.08	0.375 ±0.08	0.003 *
Pi root content (%)	0.324 ±0.06	0.374 ±0.06	0.352 ±0.06	0.308 ±0.06	0.382 ±0.06	0.322 ±0.06	0.357 ±0.06	0.307 ±0.07	0.0021 *

Babylon and Latifya showed great root fresh weight were 0.529, 0.530 and 0.580g, whereas Ibaa99 showed lowest weight 0.181g (Fig. 2).

Pi Acquisition

The Pi content of wheat differ significantly (P≤0.05) and gradually decreased under P-deficiency stress and deprivation. Plants grown under Pi deficit and deprivation in hydroponics had significantly lower total shoot P concentrations than plants grown in the adequate of Pi,

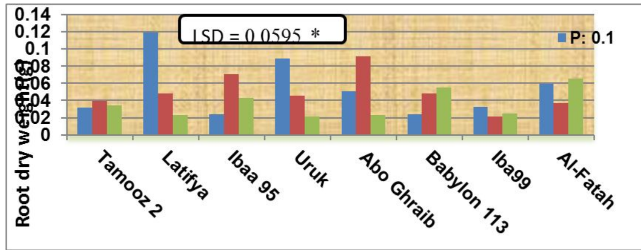

Fig. 2: Mean values for root length of genotypes under Pi treatments.

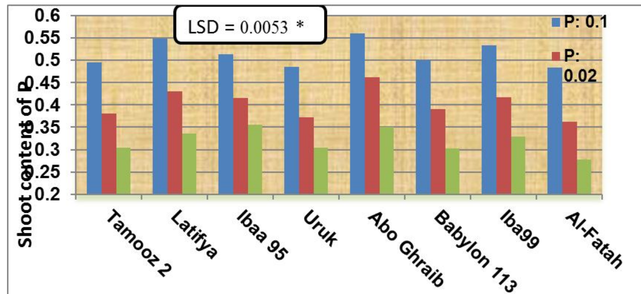

Fig. 3: Mean values for shoot P content of genotypes under Pi treatments.

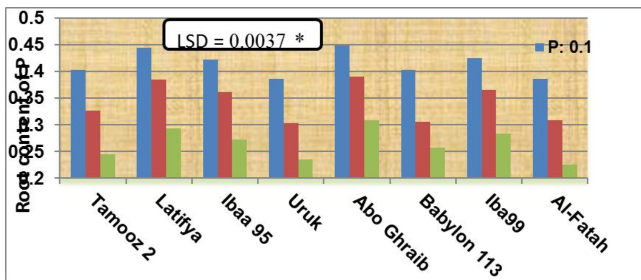

Fig. 4: Mean values for root P content of genotypes under Pi treatments.

the reduction was 21.55 and 37.86% under deficit 0.02 *mM* and deprivation 0 P, respectively compared with adequate 0.1 *mM* (Table 4). Based on the results of the present study, the phosphorus content in roots exhibited a behavior similar to the shoots content of phosphorous. The control treatment (0.1*mM* P) gave the highest phosphorous content, it decreased gradually and significantly (P≤0.05), with an increase in phosphorus stress (0.02*mM*) and deprivation (0 P) by 17.35 and 36.14%, respectively (Table 4). Total P concentration in the shoot was also measured in genotypes grown under P levels conditions. Maximum shoot P concentration was observed in Genotype AbuGhraib which gave 0.457% compared to AL-Fatah which gave 0.375%. The rest of the genotypes had values ranging between these mean (Table 5). The phosphorus content in the roots of Latifya and AbuGhraib genotypes had higher content of P which gave 0.0.374 and 0.382% compared to AL-Fatah which gave the lowest content was 0.307% (Table 5).

Uptake and translocation of phosphorus in the wheat plants under P level conditions in the medium was analyzed, The total P concentration in the shoot of Latifya and AbuGhraib genotypes were the highest (0.548 and0.560 %) and 0.453 and 0.462%, respectively at an adequate level (0.1*mM*P) deficit 0.02*mM*), as were the AbuGhraib and Ibaa95 genotypes were the highest (0.350 and 0.356 %), respectively, at the deprivation. While the genotypes Uruk and AL-Fatah had minimal P content (0.486 and 0.278%), at adequate and deprivation, respectively and Uruk 0.373% at deficit 0.02*mM*) (Fig. 3). Total P concentration in the roots was also measured in genotypes grown under P levels conditions. The genotypes Latifya and AbuGhraib had higher content of P which gave 0.445 and 0.449 % and 0.382,0.385% compared to those genotypes AL-Fatah and Uruk which gave 0.386 and 0.386% at adequate P supply (0.1*mM* P) and Uruk (0303% at deficient P level (0.02*mM* P). At deprivation P level the maximum root P concentration was observed in genotypes AbuGhraib with content of 0.309%, whereas maximum reduction in root P concentration because of P deprivation, was observed in genotypes AL-Fatah Uruk with content of 0.226 and 0.235 %, respectively (Fig. 4).

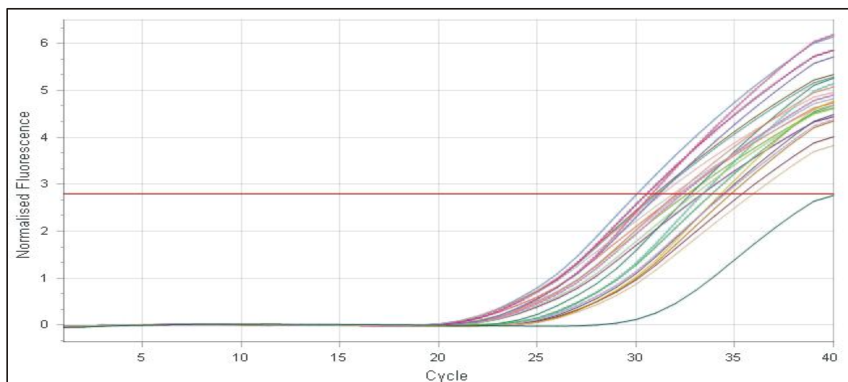

Fig. 5: The Amplification Plots of Housekeeping Gene *16SrRNA*.

Two genes *TaPht1* and *TaPHO2* expression was measured in roots of wheat under conditions of P deficit (0.02*mM*), P(0) deprivation and control (P adequate 0.1*mM*) plants using quantitative RT-PCR one step (qRT-PCR) with reference gene

Table 6: Fold of *TaPHT1* gene expression depending on $2^{-\Delta\Delta Ct}$ Method.

P levels (*mM*)	Genotype	*TaQhnRNP*	*TaPht1*	ΔCT	ΔΔCT	Folding
0.1	1Ibaa99	33.31	24.60	-8.7	0.0	**1.0**
	Uruk	32.57	25.62	-6.9	0.0	**1.0**
	Ibaa95	32.12	24.32	-7.8	0.0	**1.0**
	AbuGhraib	31.04	26.36	-4.7	0.0	**1.0**
	Latifya	30.93	22.28	-8.6	0.0	**1.0**
	Tamooz2	31.28	24.78	-6.5	0.0	**1.0**
	Babylon	30.02	22.19	-7.8	0.0	**1.0**
	AL-Fatah	30.51	23.65	-6.9	0.0	**1.0**
0.02	1Ibaa99	35.34	26.65	-8.7	0.0	**1.0**
	Uruk	34.27	23.80	-10.5	-3.5	**11.5**
	Ibaa95	34.40	24.04	-10.4	-2.6	**5.9**
	AbuGhraib	30.78	23.64	-7.1	-2.5	**5.5**
	Latifya	37.08	32.89	-4.2	4.5	**0.05**
	Tamooz2	32.17	24.99	-7.2	-0.7	**1.6**
	Babylon	32.75	25.20	-7.5	0.3	**0.8**
	AL-Fatah	34.47	22.43	-12.0	-5.2	**36.3**
0	1Ibaa99	34.78	29.26	-5.5	3.2	**0.1**
	Uruk	32.00	28.99	-3.0	3.9	**0.1**
	Ibaa95	33.81	24.40	-9.4	-1.6	**3.1**
	AbuGhraib	32.31	28.22	-4.1	0.6	**0.7**
	Latifya	31.84	25.16	-6.7	2.0	**0.3**
	Tamooz2	31.14	25.54	-5.6	0.9	**0.5**
	Babylon	32.95	24.74	-8.2	-0.4	**1.3**
	AL-Fatah	35.89	25.78	-10.1	-3.3	**9.5**

(*TaQhnRNP*) for normalization the results with using standard deviation for error estimation in $2^{-\Delta\Delta CT}$ values. The results of RT-PCR one step calculated as fold-differences which is relative quantification ($2^{\Delta\Delta Ct}$) after calculating the ΔΔCT values. No significant differences were found between the genotypes under P levels conditions with respect to Ct for reference gene *TaQhnRNP* which ranged from 30.02 to 35.89. The pattern of amplification of the gene was shown in the fig. 5.

The expression patterns of *TaPht1* gene was detected under the normal growth conditions. After normalization, No root expression of *TaPht1* was detected in hydroponically grown plants of 8 wheat genotypes (Fig. 6). Expression analysis revealed major differences among the 8 genotypes and their responses to Pi –low level (0.02*mM*). The transcripts of the wheat *TaPht1* gene exhibited largely variation. According to the normalized expression intensity, the expression levels of the gene was classified into five groups: high expression level group include Uruk and AL-Fatah (11.5 and 36.3 folds), mid-expression level group include Ibaa95 and AbuGhraib (5.9 and 5.5 folds), low expression level group represented by Tamooz2 (1.6 fold), dowexpression level group include Latifya and Babylon (0.05 and 0.8 folds) and no root expression group represented by Ibaa99 (1 fold) (Table 6). Once exposed to Pi deprivation *TaPht1* gene altered its expression levels (Fig. 6), suggesting that a large set of wheat genes are regulated by the Pi- deprivation signaling, the expression levels of the gene was classified into four groups: The expression of *TaPHT1* did not show a response to P deprivation in the roots of Ibaa99 and Uruk genotypes (1 fold).

The gene had low values in P- deprivated root of Babylon genotype (1.3 fold), while had highest values in P-deprivated roots of AL-Fatah genotype (9.5 fold) and mid-expression in roots of Ibaa95 genotype (3.5 fold). Downregulated of the gene was in the roots of AbuGhraib, Latifya and Tamooz2 (0.7, 0.3 and 0.5 folds), respectively (Table 6).

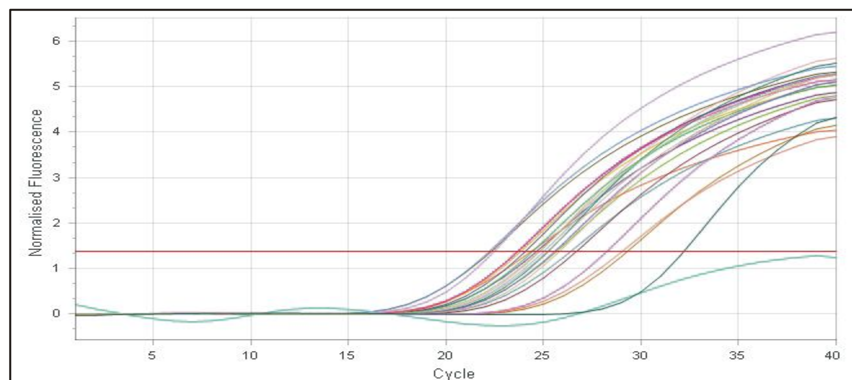

Fig. 6: The amplification plots of *TaPHT1* gene.

A quantitative real-time PCR (qRT-PCR) analysis was used to investigate the expression pattern of *TaPHO2* gene in different wheat genotypes under identical conditions. To investigate whether 8 Iraqi genotypes presented differences in the P signaling and homeostasis pathway, gene expression of *TaPHO2* was analysis, all genotypes exhibited gene expression in different degrees when exposed to P-low level (0.02*mM*) (Fig. 7). Interestingly a 119-fold higher induction was observed in Latifya followed by a 30 folds high induction was observed in AL-Fatah,

Table 7: Fold of *TaPHO2* gene expression depending on $2^{-\Delta\Delta Ct}$ Method.

P levels (*mM*)	Genotype	*TaQhnRNP*	*TaPHO2*	ΔCT	ΔΔCT	Folding
0.1	1Ibaa99	33.3	22.6	-10.7	0.0	**1.0**
	Uruk	32.6	23.0	-9.6	0.0	**1.0**
	Ibaa95	32.1	23.4	-8.7	0.0	**1.0**
	AbuGhraib	31.0	23.3	-7.8	0.0	**1.0**
	Latifya	30.9	22.9	-8.0	0.0	**1.0**
	Tamooz2	31.3	23.1	-8.2	0.0	**1.0**
	Babylon	30.0	22.5	-7.5	0.0	**1.0**
	AL-Fatah	30.5	23.3	-7.2	0.0	**1.0**
0.02	1Ibaa99	35.3	22.7	-12.7	-2.0	**3.9**
	Uruk	34.3	22.6	-11.7	-2.1	**4.3**
	Ibaa95	34.4	22.5	-11.9	-3.2	**9.1**
	AbuGhraib	30.8	22.5	-8.3	-0.5	**1.4**
	Latifya	37.1	22.2	-14.9	-6.9	**119.0**
	Tamooz2	32.2	22.3	-9.9	-1.7	**3.2**
	Babylon	32.7	21.9	-10.8	-3.3	**10.1**
	AL-Fatah	34.5	22.4	-12.1	-4.9	**30.0**
0	1Ibaa99	34.8	23.2	-11.6	-0.9	**1.9**
	Uruk	32.0	23.8	-8.2	1.3	**0.4**
	Ibaa95	33.8	22.6	-11.2	-2.5	**5.6**
	AbuGhraib	32.3	23.5	-8.8	-1.0	**2.0**
	Latifya	31.8	22.6	-9.2	-1.2	**2.3**
	Tamooz2	31.1	22.0	-9.1	-1.0	**1.9**
	Babylon	33.0	21.8	-11.2	-3.7	**12.6**
	AL-Fatah	35.9	22.8	-13.1	-5.9	**57.9**

also Ibaa95 and Babylon exhibited mid-expression levels 9.1 and 10.1 folds, respectively. While Ibaa99, Uruk, AbuGhraib and Tamooz2 had low expression levels 3.9, 4.3, 1.4, 3.2 folds (Table 7). Under Pi deprivation, *TaPHO2* gene altered its expression levels (Fig. 7). Expression of tested gene in genotypes roots was influenced by applied P levels. A response with up-regulated transcript levels for *TaPHO2* was expressed strongest to 57.9-fold in AL-Fatah and then declined to 12.6 folds in Babylon and declined again to low expression with an 1.9, 1.9, 2.0, 2.3 and 5.6 folds in Ibaa99, Tamooz2, AbuGhraib, Latifya and Ibaa95 genotypes, respectively. In contrast, *TaPHO2* showed inhibitory responses to P starvation in Uruk genotype and was down-regulated to 0.4 fold (Table 7). Interestingly the two genes (*TaPht1* and *TaPHO2*) showed down-regulation to 0.1 and 0.4 fold, respectively in Uruk genotype when exposed to P deprivation (Tables 6, 7).

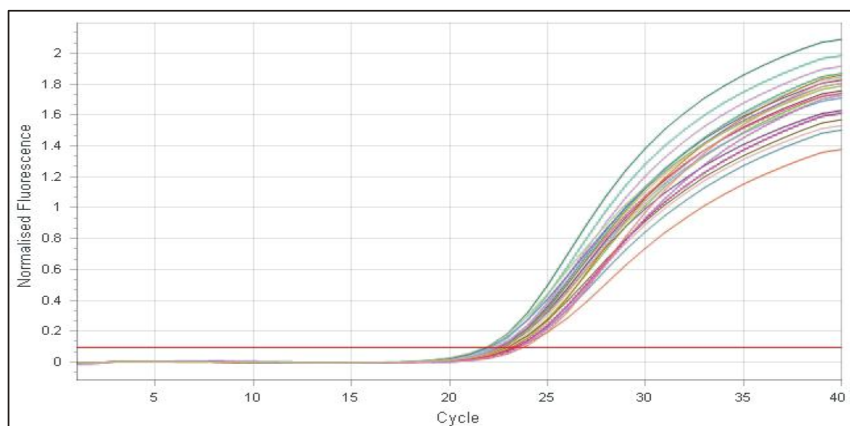

Fig. 7: The amplification plots of *TaPHO2* gene.

Discussion

Shoot and root dry weight

A low-P or P deprivation environment has a significant impact on the growth of plants (Shukla *et al*., 2016). As expected, significant differences in dry weight of shoots and roots were observed under adequate and deprivation Pi conditions when grown hydroponically (Table 4). Significant variation in shoot and root dry weight at deficient and adequate P levels were also reported by many researchers (Bilal *et al*., 2018; de Souza Campos *et al.,* 2019; Nguyen and Stangoulis, 2019; Wang *et al*., 2019). However, under low-Pi and Pi deprivation, plants showed a clear reduction in shoot growth ($P \leq 0.05$) by 51.23 and 63.86%, respectively and by 29.41% for root at deprivation compared with plants growing under adequate Pi conditions. The results show that root growth of plants was comparatively less inhibited than shoot growth under P deficient conditions (Table 4). Root dry matter was significantly correlated with shoot dry matter and shoot P uptake indicating the importance of root growth in relative tolerance of plants against P deficiency (Aziz *et al*., 2011). The crop species and genotypes of different species have varied responses under various supply of P (Aziz *et al*., 2015), in this study, wheat genotypes also showed differential responses to various P concentrations. The effect was more severe in the genotype Ibaa99, with a 53.70% reduction in dry shoot biomass reduction from Latifya genotype who exhibited higher biomass. Similar trend was observed for roots, AL-Fatah produced higher wet root. The effect was more severe in Ibaa99, with a 52.73%, reduction in dry root biomass from AbuGhraib who exhibited higher

biomass (Table 5). The findings are consistent with various previous findings, Gajri and Prihar, (1985) suggested that increases in RDW and RLD may be associated with increases in wheat development and growth. Moreover, the increase in P acquisition in P-efficient genotypes might be due to an increase in the root absorption area, which may also affect the ability of plants to acquire nutrients from the medium (Hammond and White, 2008).

Some genotypes such as AbuGhraib and Babylon had higher dry weight under conditions of phosphorous deprivation than some genotypes like Ibaa95 and Ibaa99 that received adequate quantities of phosphorous (Fig. 4). Therefore, SDM can be an appropriate selection criterion for evaluating cultivar P use efficiency at early growth stages of wheat crop. The same criterion has effectively been used by other researchers (Ozturk *et al.,* 2005; Abbas *et al*., 2016; Bilal *et al*., 2018) Interestingly, under these conditions, Babylon showed smaller losses of shoot biomass production and greater increments in root growth (Fig. 4) which can grow better under both P-deficient and -sufficient conditions is desired because it can be successfully grown in a low P level. Mostly, for screening P-efficient genotypes, high dry matter production under P-deficient conditions is the most important trait (Osborne and Rengel, 2002).

Pi Acquisition

Phosphorus acquisition efficiency of wheat genotypes was investigated by applying a technique hydroponic system, 8 wheat genotypes were compared under three P levels. The results showed that their growth was obviously inhibited by the low P level and deprivation and the symptoms of P deficiency varied between genotypes. It is noticed that plant P concentration differed between genotypes under the low-P level and deprivation, the Pi content of genotypes decreased by 21.55 and 37.86% in shoots and 17.35 and 36.14% in root, respectively (Table 4). Meanwhile, the Pi content of Genotype AbuGhraib was always higher than that of genotype AL-Fatah (Table 5), suggesting that the phosphate uptake and transportation efficiency of AbuGhraib was higher than those of AL-Fatah. Better P acquisition and utilization under P stressed environment were reported in various genotypes of wheat (Kosar *et al*., 2003; Aziz *et al*., 2014; Bilal *et al*., 2018).

Genotypes showed marked differences in P content at low, deprivation and adequate levels of P. It is interesting to note that P content in shoots at low P level and deprivation differed significantly (Fig. 3). This indicates that wheat genotypes which accumulated more P in their shoots from a deficient growth medium were more tolerant to P deficiency stress. Yaseen *et al*., (2008) also observed similar responses. This differential P content had close link with differences in P uptake of roots which were mainly associated with the differences in root P concentration (Fig. 4). Deprivation of P also reduced the total P concentration in the shoot of all genotypes. In the shoots, the effect was statistically significant for Al-Fatah (Fig. 3). Besides the high total P concentration in the shoot at P adequate, Latifya and AbuGhraib kept the Pi levels were higher at deprivation (Fig. 3), which suggests a stronger control over the P partition. Increased remobilization of absorbed P among various tissues within plants under P starvation might be a mechanism for better P efficiency in cultivars differing in P acquisition and utilization (Irfan *et al.*, 2020). Significant variation for P absorption and utilization among crop species and even genotypes within the same species is well documented (Akhtar *et al*., 2016; Irfan, *et al*., 2017; Abbas *et al*., 2018).

Roots are critical for plant growth and are directly exposed to the soil environment. A variation in root P content under varying levels of P among different wheat genotypes was observed in this study (Fig. 4). As P mobility in soil is limited, higher plant root growth and changes in root morphology is helpful for more P uptake. At deprivation P level the maximum root P concentration was observed in genotypes AbuGhraib, whereas maximum reduction in root P concentration because of P deprivation was observed in genotypes AL-Fatah and Uruk (Fig. 4). In order to enhance P acquisition under limited P supply situations, plant often modifies root morphological traits to increase the ability of root to absorb P from soil (Schjorring and Nielsen, 1987). Larger root system provides greater adsorption surface for soil nutrients, which is particularly important for soil P as a less mobile ion (Singh Gahoonia and Nielsen, 2004).

Gene expression

The assumption in the use of housekeeping genes in molecular studies is that their expression remains constant in the cells or tissue under investigation (Reboucas, 2013). The accuracy of qRT-PCR results is largely dependent on the selected reference genes (Huang *et al*., 2013) the validity of which is a prerequisite for the correct application of qRT-PCR to analyze changes in target gene expression (Liu *et al*., 2016). Real time PCR one step was used to determine the expression of two genes (*TaPht1* and *TaPHO2*) with *TaQhnRNP* gene used as a reference housekeeping gene to normalize the data. Many previous studies (Long *et al*., 2010; Grun, 2015; Grun *et al*., 2018) used *TaQhnRNP* gene as housekeeping gene. The ideal reference genes should have unaltered expression levels during the entire experimental process

and with proper expression intensity (Xu *et al*., 2015). No significant differences were found between genotypes under P-levels conditions with respect to Ct for reference gene *TaQhnRNP* (Fig. 5). A stable reference gene is a prerequisite for improving the reliability of qRT-PCR results. The selection of reference genes was once mainly based on the functions of housekeeping genes (Zhao *et al*., 2018). Regulation of gene expression in eukaryotes such as plants is very complex. However, a little knowledge about controlling process can open the doors to broaden our understanding on different plant characteristics (Gatehouse, 1997). The need to drive efficiency in global agricultural production has led to the illustration of a number of key genes in breeding for phosphate efficient crops (Milner *et al*., 2018). Wheat has 16 phylogenetically distinct Pi transporters, seven of them being induced by Pi deprivation (Grun *et al*., 2018). In this study, the expression profile of *TaPht1* was analysis. It is the most highly expressed and it was described as a fast responsive Pi marker. (Grun *et al*., 2018). The expression analysis revealed major differences among the genotypes and their responses to Pi deprivation (Fig. 6). All adaptive responses that plants have evolved to cope with Pi deficiency are regulated through P signaling and homeostasis mechanisms, which begin with the integration of the information of the extracellular Pi concentration and its levels in the different organs (Puga *et al*., 2017).

Several studies correlate higher Pi accumulation and, in most cases, plant growth with higher expression of Pi transporters of the *PHT1* family (Ham *et al.*, 2018). Recently, it has been shown that under Pi starvation, transcript levels of the gene *TaPht1* were the most abundant of all the *PHT1* transporter genes described in wheat (Grun *et al*., 2018). In the artificial hydroponic growth system used here, there was a rapid induction of *TaPht1* transporter in some genotypes, whereas others exhibit a down induction to Pi deprivation or no response at all. Similar observations were made for *Pht1* transporters in other plant species (Nagy *et al*., 2006; Morcuende *et al*., 2007; Lapis-Gaza *et al*., 2014).

When 8 genotypes were compared in relation to *TaPht1* expression, a very different behaviour was observed (Table 6). At P deprivation, Ibaa95 and AL-Fatah genotypes increased the relative expression of this gene when compared with P adequate, although the relative expression was three times greater for Ibaa95 and nine times for AL-Fatah. Also at P deficit (0.02mM) Uruk, Ibaa95, AbuGhraib and AL-Fatah genotypes increased the relative expression of this gene when compared with P adequate (Table 6). The results indicate that the genotype AL-Fatah was characterized by the highest expression under conditions of deficit (0.02*mM*) and deprivation (0)of phosphorous (Table 6 and Fig. 6). Nevertheless, at deprivation Babylon genotype and at deficit Tamooz2 showed a small increase in the gene expression (1.3 and 1.6 folds), respectively (Table 6).

In general, broad responses to stresses, such as P pathways, depend on the stress extent and magnitude. The main players are transcription factors, which can affect the expression of several genes. Starvation, are controlled by one or more transduction (Espindula *et al*., 2009). In the current study, expression of *TaPHT1* in the roots of Ibaa99, AbuGhraib, Latifya and Tamooz2 genotypes was inhibited by P deprivation. Teng *et al.,* (2017) and Deng *et al*., (2018) reported that the expressions of *TaPHT1.2, TaPHT1.1/1.9* and *TaPHT1.10* were root-specific and were lower when the P supply was low rather than high. Phosphate starvation responses influence root Pi uptake mechanisms, as well as Pi partitioning between roots and aerial tissues, via altered *Pht1* transporter expression (Grun *et al.,* 2018).

Latifya and AbuGhraib were less affected by Pi deprivation, presenting higher biomass production and an enhanced root development andmost efficiency parameters under this condition. Although, the decrease in the genetic expression of the *TaPHT1* gene under the deprivation conditions, these two genotypes had high morphological and physiological performance and outperformed the rest of the studied genotypes and this may be associated with another group of genes. Therefore it is necessary to study a wide range of genes to know which of these genes is associated with this high superiority.

The crucial roles of *PHO2* in regulating Pi signaling have been described in *Arabidopsis* and rice and *PHO2* exists in single copy in these diploid plant species (Bari *et al*., 2006; Hu *et al*., 2011). Differences in gene expression were observed for *TaPHO2* (Table 7). It was previously shown that this transporter is regulated post-transcriptionally by the action of *PHO2* (Huang *et al*., 2013). As common wheat is an allohexaploid which contains three homoeologous genomes 28, Ouyang *et al*., (2016) identified three *TaPHO2* genes in wheat that are involved in P uptake and translocation of P. *PHO2* has been demonstrated to regulate *PHO1* and *PHT1* transporters at post-translational level (Huang *et al*., 2013). *TaPHO2* exhibited much higher expression abundance in all genotypes when exposed to P-low level (0.02*mM*) than at deprivation (0) (Fig. 7). Latifya showed 119 folds and AL-Fatah 30 folds at P-low level, In contrast

with these results, the overall expression of *TaPHO2* in roots was reduced to 57.9 and 12.6 folds exhibited by Latifya and Babylon, respectively at deprivation. In consist with these results; the overall expression of *TaPHO2* in roots was reduced again in other genotypes at both P levels except in Uruk genotype which exhibited down-regulation to 0.4 fold at deprivation (Table 7). Higher *PHO2* levels under Pi starvation in barley correlated with a higher root: shoot ratio (Huang *et al.,* 2011). Conversely, wheat plants blocked at *TaPHO2* showed a lower root: shoot ratio (Ouyang *et al*., 2016).

Results of current study revealed that Pi concentrations in shoot and roots negatively correlated with the expression of *TaPHO2*. In diploid plant species such as Arabidopsis and rice, loss of function of *PHO2* has been found to inhibit plant growth, possibly caused by the over-accumulation of Pi in shoots (Hu *et al.,* 2011). These results suggest that *PHO2* is essential to maintain Pi homeostasis and hence plant growth. Ouyang *et al.* *(*2016) found that a severe reduction in *PHO2* expression could also impair Pi homeostasis and plant growth in wheat.

Both genes *TaPht1*and *TaPHO2* exhibited decline in expression in Uruk genotype at deprivation, they showed down-regulation to 0.1 and 0.4 fold, respectively (Tables 6, 7), this indicate that may be there is another set of genes that regulates the response to phosphorous deprivation. Hamburger *et al.,* (2002) identified *Pho1*, in *A. thaliana*, which encodes a protein involved in the control of P transport to xylem. The identification of homologs in wheat would be of great assistance in dissecting this response, but this is a hard task: in *A. thaliana* there are 11 members of the *PHO1* family andonly two of them are involved in xylem loading (Stefanovic *et al*., 2007).

Conclusions

Results showed existence of genetic differences among wheat genotypes with regard to P absorption and utilization. Plant growth and development require different Pi transport processes, which need to be regulated depending on demand and Pi availability. This process requires an up-regulation of specific genes like *TaPht*1 and *TaPHO2* to enable P homeostasis. The expression patterns of *TaPht*1 gene is important for Pi acquisition, whilst others are likely to be required for Pi translocation like *TaPOH2* gene which seems to be only partly regulated by external Pi availability. Some genotypes were identified as either having high P deprivation tolerance or high P responsiveness. The findings of this study reveal that AbuGhraib and Latifya are efficient and responsive genotypes, which have potential for better growth in P limited environments. However, these results should be confirmed under field conditions.

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