Molecular depiction of efficient wheat (*Triticum aestivum* L.) genotypes under diverse phosphorus treatments

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Abstract: Six wheat (*Triticum aestivum* L.) genotypes (best, poor and control) were selected for the identification of genes responsible for better utilization of phosphorus. Molecular characterization of selected genotypes indicates that *Triticum aestivum* phosphorus transporters *TaPHT1; 4* and *TaPHT2; 1* were present in both phosphorus efficient and inefficient genotypes. Differential expression of these *TaPHTs* might be responsible for phosphorus use efficiency under phosphorus deficient conditions. To assess the polymorphism phylogeny of *TaPhTs* was constructed. Gene specific primers were used to identify the phosphorus transporters in these genotypes. The present study will be helpful to improve wheat cultivars for phosphorus deficient conditions and data can be used in gene-assisted wheat breeding.

[Muhammad Junaid, Shamroz Khan, Rehman Wali, Muhammad Arshad, Naveed Riaz, Fariz Tehreem, Ushna Mariam. **Molecular depiction of efficient wheat (*Triticum aestivum* L.) genotypes under diverse phosphorus treatments.** *Nat Sci* 2018;16(11):80-84]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 11. doi:10.7537/marsnsj161118.11.

Key words: Phosphorus, wheat, phylogeny, primer

1. Introduction

Bread wheat (*Triticum aestivum* L.) belongs to *Poaceae* (grass) family. Major worldwide efforts are to increase wheat production by using genetic resources, analysis of important yield contributing traits and genetic diversity. Wheat belongs to *Hordeae* tribe and *Triticum* genus, consist of three sub genomes named as A,B and D genome. Number of chromosomes in each genome set is seven. Genome A and B are different but homologue to each other but D genome is present only in hexaploid wheat (Huang *et al.*, 2003). Wheat have 3 distinct groups based on chromosomes number i.e. 2n=14, 2n=28 and 2n=42. These groups are called diploid, tetraploid and hexaploids respectively. Presence or absence of genome A.B and D are the bases of ploidy level in wheat. Genome A belongs to *Triticum monococum,* B and D belongs to *Aegilops speltoides* and *Aegilops tauschii* respectively (Dvorak and Akhunov, 2005).

Bread wheat consists of AABBDD genomes that were evolved in two separate events of amphiploidization. Inter-specific hybridization in *Triticum monococum* (AA) and most probably the other one is *Aegilops speltoides* (BB) followed by spontaneous doubling of chromosome resulted in *Triticum turgidum* with chromosome number 2n=4x=28 and AABB genomes. Hybridization between *Triticum tauschii* (AABB) and *Triticum turgidum* (DD) followed by doubling of chromosome resulted in evolution of *Triticum aestivum* L. with chromosome number 2n=6x=42 with AABBDD genomes. Twenty one chromosomes of bread wheat are classified in seven groups. In a group one partially homologue chromosome from each genome is placed, so each group consists of three chromosomes (Faris *et al.*, 1999). Hexaploid wheat *Triticum aestivum* comprises a large genome of 17.1 GB (Brenchley *et al.*, 2012). Wheat is a dominant cereal crop of developing countries including Pakistan. In Pakistan wheat is cultivated in rain fed and irrigated areas. It is used not only for food but also as feed and industrial material, as it is an important crop for food and feed in Pakistan. In Pakistan wheat is on top in all crops grown with respect to crop production and area under cultivation. It contributes 2% in GDP of Pakistan. Wheat grown area and production was 9.26 million hectare and 25.48 million tons respectively in cropping year 2025-16 (Pakistan Economic Survey, 2015-16).

Wheat is superior over rest of the cereals due to its unique dough formation property. Gluten protein is responsible for this extraordinary property which empowers bread to tie together. Proteome and Transcriptomic studies reported over 1125 individual components and 30,000 grain development genes respectively which have role in dough formation (Skylas *et al.*, 2000). Several biotic and abiotic reasons are associated with plant physiological changes. Wheat yield is affected substantially by several stresses and phosphorous deficiency is one of them. In macronutrients, phosphorous is important because of its association with high productivity as it plays vital role in photosynthesis, storage and transfer of energy, cell division, respiration and other mechanisms in

plant like it helps seedlings and roots to develop rapidly, improve winter hardness, promotes uniform and early heading, seed formation and seed quality and improves water use efficiency as well (Ali *et al.*, 2014). Phosphorus natural resources are depleting quickly but utilization is increasing at an annual rate of 3%. Only Morocco is contributing approximately 40% of the world's production.

A recent study summarized that phosphorous natural resources will deplete by 2050 (Gaxiola *et al.*, 2011) and the alternate means will be insufficient to overcome this gap. Different gene families (*TaPhTs*) are associated with phosphorus uptake and utilization efficiency in wheat which express in limited phosphorus availability, like *TaPHT1;4* express under phosphorus deprivation conditions in root area and increase the soil phosphorus uptake (Liu *et al.*, 2013a). *TaPht2;1* express in leaves and involves in phosphorus translocation so it is involve in efficient utilization of available phosphorus in wheat (Guo *et al.*, 2014). Advance molecular techniques can be deployed to develop varieties with high phosphorous efficiency and better performance.

2. Materials and methods

The seed for fifty different wheat varieties and lines were collected from Cereal Research Program, University of agriculture Faisalabad, Pakistan. Table 1 denotes genotypes used in this study. These fifty wheat genotypes were sown in field in three different plots containing different Phosphorus levels (P0, P100 and P200 mg/Kg soil). Total available phosphorus was determined using sodium bicarbonate method (Olsen *et al.,* 1954). Factorial randomized complete block design was followed with three repetitions. At appropriate time data of these trait were recorded. The traits were plant height, flag leaf area, peduncle length, spike length, productive tillers per plant, spikelets per spike, spike weight, number of grains per spike, 1000-grain weight, biological yield per plant, grain yield per spike, grain yield per plant and harvest index.

Table 1 Wheat genotypes

Sr. NO	Genotype	Sr. NO	Genotype
1	MF-1	26	SULEMAN 96
2	MF-2	27	SHAHEEN 94
3	MF-3	28	KOHSAR 95
4	MF-4	29	BAKHTAAWAR 93
5	MF-5	30	9867
6	NAX1 5020-7	31	CHAKWAL 86
7	NAX1 5020-27	32	CHAKWAL 50
8	NAX1 5907	33	1031
9	NAX2 5004	34	KRICHAUFF
10	NAX2 5042	35	1255
11	NAX2 5924	36	WAL 23
12	KHARCHIA 65	37	UJALA
13	STW 139	38	PUNJAB 11
14	STW 135	39	978
15	SEHAR 2006	40	ZA-1
16	MANTHAR 2003	41	GALAXY
17	IQBAL 2000	42	AAS 11
18	INQLAB 91	43	ZA-2
19	LU 26	44	ZA-4
20	PASBAN 90	45	LINE 1
21	FAISALABAD 83	46	9494
22	KOH E NOOR 83	47	LINE 2
23	ROHTAS 90	48	34ESWYT-13-14/146
24	WATAN	49	34ESWYT-138
25	NOSHEHRA 96	50	ZA-6

After screening of these genotypes on the basis of yield and yield related parameters six genotypes were selected. Selection of phosphorus efficient genotypes was made on the basis of grain yield per plant. Two standard cultivar Punjab-2011 and Galaxy-2013, three phosphorus efficient genotypes Krichauff, Manthar-2003, ZA-6 was selected on the basis of productive tillers per plant under low phosphorus and one phosphorus inefficient genotype MF-1 was selected Phylogenetic tree based on the phosphorus

transporter genes CDS (coding DNA sequences) were obtained from Phytozome database, of *Arabidopsis*, *Brachypodium,* rice, maize and wheat genes. The tree was inferred after sequence alignment by CLASTAL-Omega (Sievers *et al*., 2011) using the Neighbour-Joining method (Saitou and Nei, 1987) and visualized in topology-only mode. Only bootstrap values >50%, as calculated from 1,000 replicates, are shown. Phylogenetic analyses were conducted in MEGA6 (Tamura *et al*., 2013). A diamond marker indicates *Arabidopsis*, a triangle indicate rice, a circle indicates maize, and filled rectangle indicate wheat and hollow rectangle indicate *Brachypodium* genes. For gene abbreviations, see text.

2.1 Molecular characterization

Phosphorus transporter genes *TaPHT1;4* and TaPht2;4 are involve in phosphorus uptake and utilization. *TaPHT1;4* involve in phosphorus uptake and its expression takes place in root in case of limited phosphorus availability (Liu *et al.*, 2013a), and TaPht2;4 is involved in re-allocation and efficient utilization of phosphorus and its expression takes place in leaves (Guo *et al.*, 2014). These are the gene that may present in wheat genotypes which are the efficient in phosphorus use efficiency under limited phosphorus availability. Sequences of above mentioned gene were downloaded from NCBI (https://www.ncbi.nlm.nih.gov). Gene specific primers were designed by using ApE (A Plasmid Editor) and AmplifX 1.5.4 software. DNA was extracted from six wheat genotypes, selected after screening of 50 genotypes at different phosphorus levels. Selection of phosphorus efficient genotypes was made on the basis of grain yield per plant. Two standard cultivar Punjab-2011 and Galaxy-2013, three phosphorus efficient genotypes Krichauff, Manthar-2003, ZA-6 was selected on the basis of productive tillers per plant under low phosphorus and one phosphorus inefficient genotype MF-1 was selected for DNA extraction. Extracted DNA was stained with ethidium bromide, run on 1% gel and seen under UV light to check its quality.

Table 2 Primers used for gradient PCR.

Sr.	Gene	Primer	Sequence	Given Temp.
1	TaPHTs1; 4	Fwd.	CGTGTGGCGCATAGTACTCAT	62.6
2		Rew.	CCATGGAGAAGAGGCCAAACT	62.6
3	TaPHTs1; 2	Fwd.	CGAAGCAAGCTACATCAGACAT	60.8
4		Rew.	CGATCTTGCTGAAGATGTCCT	62.6

3. Results and discussion

The history of evolution was incidental by the help of Neighbor - Joining scheme. The consensus of tree incidental with replicates of 1000 is collected for representation of history of evolution of taxa under studied. Branches are distorted which are matching to partitions with <50% replicates of bootstrap reproduced. Forty five sequences of nucleotide involved in analysis. All ambiguous positions for separately pair of sequence were removed. Analysis for evolution were accompanied in MEGA6. The candidate genes, which are required to identify are highlighted with red colour in phylogenetic tree. The other gene in same clad from different crops may perform similar function in respective class. The homologue gene in other clad may partially similar to these gene in their function.

Phylogenetic tree indicates the relation of candidate phosphorus transporter genes *TaPHT1;4* and *TaPht2;1* with other similar gene in other crops of same family, like rice, maize, brachypodium, and model plant *A. thaliana.* The tree shows that the gene in same clad with these phosphorus transporters are similar in function. And the gene in different clads partially similar in function to these phosphorus transporters. *TaPHT1;4* in wheat is similar to function with other genes of maize and brachypodium in same clad. *TaPht2;1* is similar in function with other crops in same clad and in different clad the homologues of this gene are present that may perform similar function to *TaPht2;1*.

Six varieties were selected on the base of field data, three phosphorus efficient and three in-efficient. Gene specific primers were used to identify the phosphorus transporters in these genotypes. The bands on right side of DNA-Ladder (L) indicates the amplification of *TaPHT1; 4* with amplicon size of 218 base pairs and bands on right side of ladder indicates the amplification of *TaPHT2; 1* with amplicon size of 179 base pairs as shown in figure 4.28.

The numbers 1, 2, 3, 4, 5 and 6 show the genotypes names as Punjab-2011, Galaxy-2013, MF-1, Krichauff, Manthar-2003 and ZA-6 respectively.

Phosphorus transporter *TaPHT1;4* and *TaPht2;1* are involved in phosphorus uptake and phosphorus utilization efficiency respectively (Liu *et al.*, 2013a; Guo *et al.*, 2014).) It is interesting to see that these phosphorus transporters were found in both efficient and in-efficient wheat varieties (figure 4.28). This suggest that only the differential expression of these phosphorus transporters is responsible for phosphorus use efficiency in wheat (Miao *at al*., 2009). In other cereal crops like rice the promotor analysis of Barley *Pht1;1* reveals that it increase the expression of

phosphorus transporter in rice that ultimately results in improved phosphorus use efficiency (Schünmann *et al.,* 2004). Members of the *TaPHT1* gene family like *TaPHT1; 1*, *TaPHT1; 2, TaPHT1; 2, TaPHT1; 9* and *TaPHT1; 10* expressed in wheat varieties under phosphorus stress and the expression level of these phosphorus transporters is different in different wheat varieties (Teng *et al.*, 2017).

Molecular characterization of selected genotypes indicates that *TaPHT1; 4* and *TaPHT2; 1* were present in both phosphorus efficient and inefficient genotypes. Differential expression of these *TaPHTs* might be responsible for phosphorus use efficiency under phosphorus deficient conditions. Marker assisted breeding generally take less time but selection for low phosphorus tolerant varieties on the basis of QTLs is limited. Transgenic plants development through genetic engineering is robust, reliable, and cost efficient strategy as the genes encoding APase, transcription factors and high affinity phosphorus transporters and protein kinases that are involve in organic acid production can be targeted in genetic engineering.

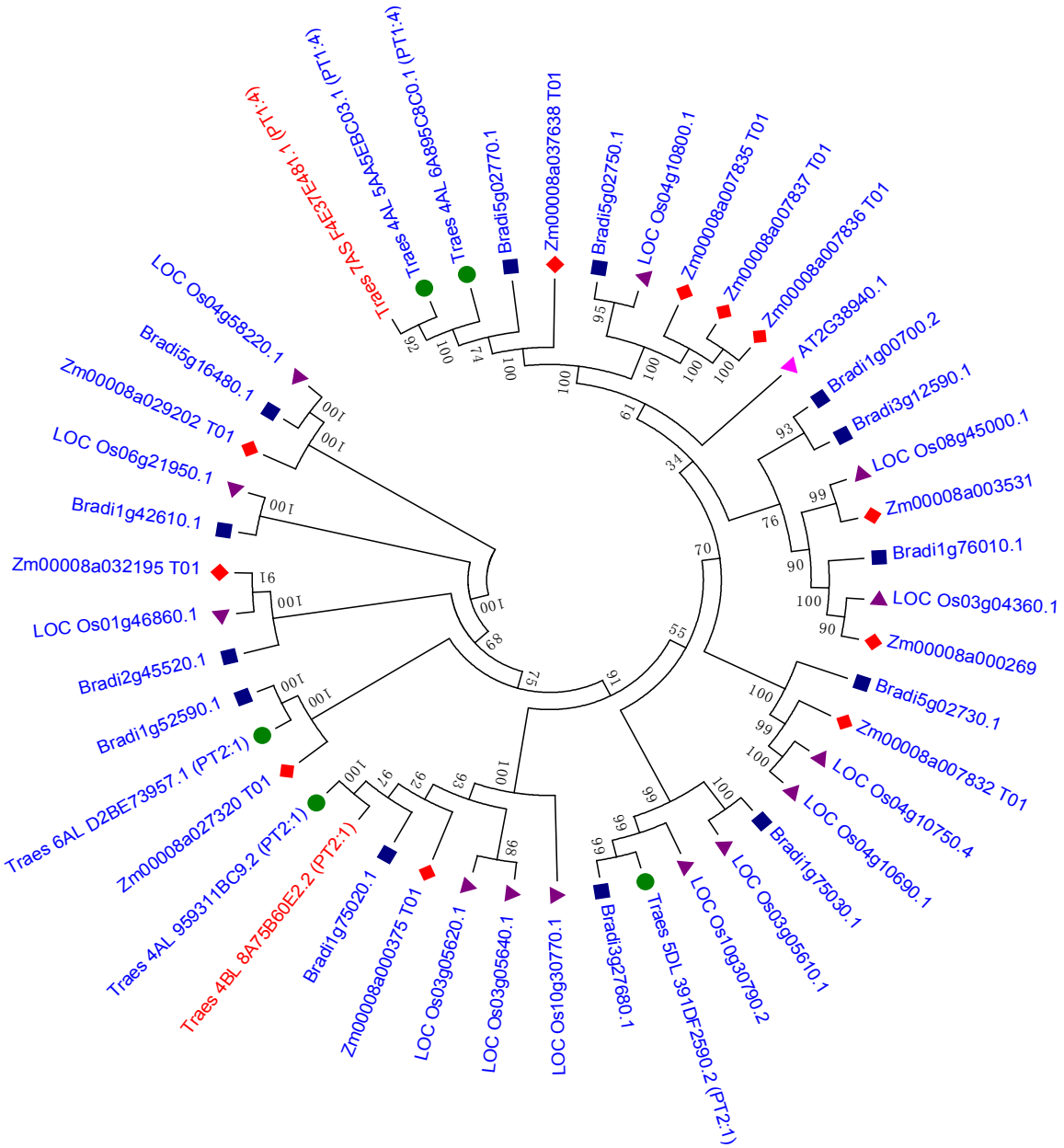

Figure. 1. Relationships of taxa by evolution

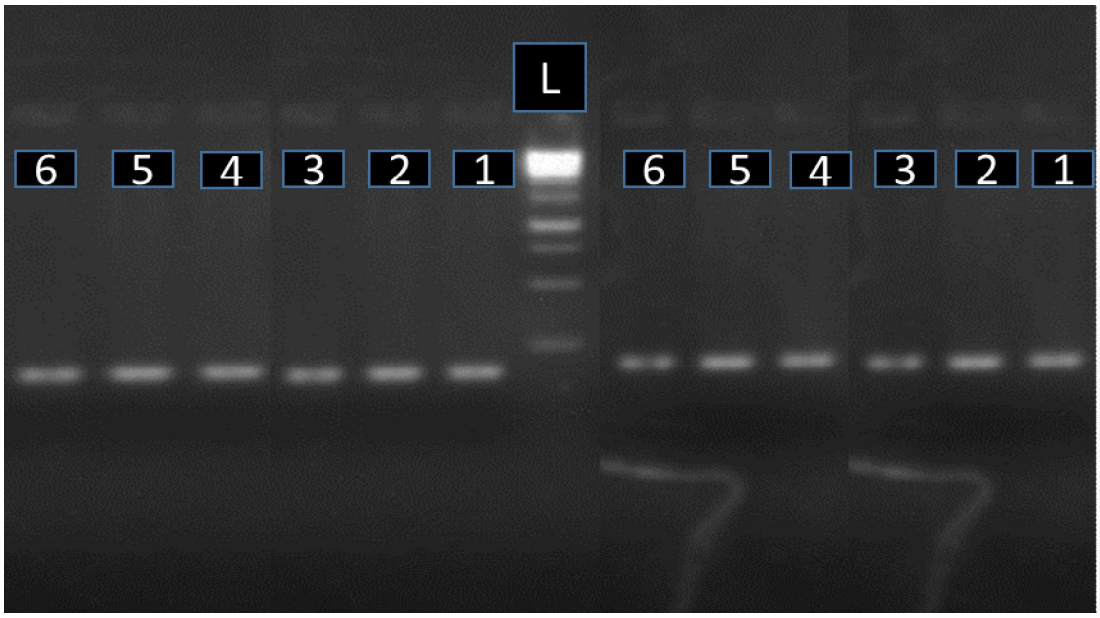

Figure: 2Amplified phosphorus transporter

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9/5/2018