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Network analysis of phosphorus stress down regulated differentially expressed genes for identification of hub genes through Cytoscape

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Abstract
Molecular responses to phosphorus deficiency have stimulated research towards the development of crops with improved phosphorus use efficiency. Transcriptome profiling data for studying phosphorus stress-responsive genes have indicated variations in levels of gene expression in wheat, but only limited studies have been carried out on co-expression network analysis for the identification of master regulators for phosphorus stress tolerance. In this study, we performed unweighted network analysis and identified hub genes for phosphorus stress tolerance from a total of 798 differentially expressed genes from publicly available transcriptomic data. Out of the total 3 modules identified from the analysis, the one with the most nodes and 260 edges was selected for the identification of hub genes. Based on highly dense interactions, we identified 14 intra-modular hub genes, leading to a total of 26 potential candidate genes. The total top 10 bottle neck genes were identified, which consist of 3 hub genes and the total top 10 genes on the basis of degree were identified through Cytohubba. The GO analysis of hub genes identified the maximum number of hub genes involved in photosynthesis and related processes. The KEGG analysis of hub genes found them to be related to photosynthesis, carbon metabolism, and fixation. Thus, our study not only provided an effective approach for studying phosphorus stress tolerance in wheat but also identified major candidate genes that can be further validated by functional genomics.

Keywords: Phosphorus, transcriptome, co-expression, network analysis, candidate genes, GO and KEGG

1. Introduction
Phosphorus (P) is one of the most essential macronutrients for plants, particularly for growth, function, and reproduction. It is necessary for respiration, photosynthesis, enzyme activity, membrane stability, and the creation of membranes (Yasmeen *et al*, 2021) [27]. It makes up 0.2-0.6% of a plant's dry weight and is essential for the production of phospholipids, nucleic acids, and ATP (Abbas *et al*, 2022) [1]. The main sources of phosphorus in plants include phospholipids, nucleic acids, phosphorylated proteins, and phosphorus esters. In processes including photosynthesis, redox, energy production, phosphorylation/dephosphorylation, and energy conversion, phosphorus takes part (Kothari *et al.*, 2023) [9]. Residual P (phosphoproteins), inorganic P (Inorganic phosphate), lipid P (phospholipids), metabolite P (such as sugar phosphates and ATP), and nucleic acid P (PN, RNA, and DNA) are the five main types of phosphorus that can be found in cereal crops (Ming *et al.*, 2000) [11]. It is estimated that P deficiency exists on around 67% of the world's cropland (Dhillon *et al.*, 2017) [5]. In tropical climates, phosphorus can form crystals of iron (Fe) and aluminum (Al), and the quantity of phosphorus that is available for plant growth is decreased by both minerals (Penn and Camberato, 2019) [14]. Due to the high reactivity of phosphorus with some metals, such as calcium (Ca), iron (Fe), and aluminum (Al), there is the formation of metal complexes, which cause between 75% and 90% of phosphorus to precipitate or adsorb in the soils. Only a very small concentration of phosphorus (0.01% of the total phosphorus) is available to plants. As a result, many agricultural soils are lacking in phosphorus (Rezakhani *et al.*, 2019) [17]. P fertilizers are applied to crops to compensate for this deficiency. However, applying phosphorus fertilizer in excess can disturb the equilibrium of nutrients within the soil. (I) This imbalance has the potential to influence the way wheat plants absorb nutrients, potentially causing shortages of other vital nutrients. This, in turn, could have repercussions for the overall health and yield of the crops (Pahalvi *et al.*, 2021) [13].

(II) The growth of the plant's root system, which is essential for water and nutrient intake, might be hampered by high phosphorus fertilizer levels. (III) The overflow of surplus phosphorus fertilizers from agricultural fields has the potential to result in water contamination.

Once phosphorus makes its way into water systems, it can stimulate the proliferation of algae and trigger a process called eutrophication (Ozlu *et al.,* 2018) [12]. (IV) When the utilization of phosphorus fertilizers is not properly optimized, it can result in diminished crop yields and decreased profitability for farmers (Krasilnikov *et al.,* 2022) [8]. Also, the non-renewable inorganic source of P fertilizers is likely to be exhausted in the near future, emphasizing the need to develop crops that may utilize phosphorus more efficiently. Phosphorus deficiency limits photosynthetic activity in plants by altering the photosynthetic phosphorylation process. ATPase and NADPH, which are essential for energy metabolism and material composition, are involved in plant photosynthesis, and the low phosphorus concentration has a direct effect on their behaviour (Wang *et al.,* 2019) [26]. Inhibition of the photosynthetic phosphorylation process can result in delayed metabolism, which can influence crop growth and development.

Wheat (*Triticum aestivum),* a plant of the Poaceae family, is the most widely cultivated cereal crop in the world. Wheat contributes to more than 30% of global cereal output and 50% of global cereal trade, according to Sharma *et al.* (2015) [20]. In terms of planted area and grain production, it is one of the most important staple crops in the world. It is also one of the most widely farmed grains. Pan wheat, also known as hexaploid wheat (2n = 6 = 42; AABBDD genomes), was produced by fusing together three diploid genomes; more than 80% of the genome (17 GB) is made up of repetitive sequences (Venske *et al,* 2019) [25]. Starch, protein, vitamins, phytochemicals, carbohydrates, fats, and fiber are all abundant in wheat. Wheat is used to make breads, pastries, pasta, noodles, and other functional items because it contains a small amount of the protein called gluten (McDonald *et al,* 2015) [10]. Wheat production is greatly influenced by multiple abiotic stresses such as drought, heat, salinity, submergence, and heavy metals. Release of the genome sequence for hexaploid wheat (genomes A, B, and D) (International Wheat Genome Sequencing Consortium, 2014), the fully annotated reference-genome assembly (IWGSC RefSeqv1.1; Appels *et al.,* 2018) [2], combined with omics approaches, has improved understanding of wheat's response to abiotic stress at the molecular level. However, very few studies have been conducted to understand the complex trait of nutritional stress tolerance in wheat.

The interaction of genes, proteins, tiny molecules, metabolites, and nucleic acids drives the complexity of organism biology. It is crucial to interpret these interactions in order to comprehend the biological system. According to the genetic code, based on the mRNAs' potential for coding, DNA is converted to RNA, which is subsequently translated into proteins. Systems biology's main goal is to understand the entire biological system by clarifying how each component behaves and how they interact with one another (Shaik *et al,* 2019) [18]. Protein expression profiles and other genomic data for a biological system are provided by these approaches in one format or another. Based on the idea that genes with similar expression profiles are more likely to interact with one another, these biological networks were developed. Comprehending the systematic approach that underlies the molecular mechanism of how cereal crops respond to phosphorus scarcity is crucial for enhancing the efficiency of phosphorus utilization. Additionally, genetic methods have revealed that tolerance to phosphate (Pi) stress is regulated by multiple genes. We can better comprehend the complicated multilocus trait and transcriptional network that cause phosphorus stress by using a systems approach. Characteristics of gene centrality can be inferred from variables including degree, proximity, and proportion. "Hub genes" are the genes that are most central (Plaimas *et al.,* 2010) [15]. One of the potential methods to handle this problem and comprehend the biology underlying the numerous mechanisms and biological processes is network analysis (Shaik *et al.,* 2019) [18].

In this study, we used 798 differentially expressed genes from the publicly available transcriptomic data (Kaur *et al,* 2021) [6] for the identification of hub genes responsible for phosphorus stress tolerance. We have focused on genes present in roots only because, based on previous research studies (Kaur *et al,* 2021; Abbas *et al,* 2022; and Juan *et al,* 2014) [1, 6, 7] on cereal plants, it has been reported that plants modify their root system architecture and increase the capacity of the transport system for acquiring phosphate (Pi) from soils as a response to phosphate stress.

2. Materials and Method

The complete work flow of network analysis for identification of hub genes and bottleneck genes from down-regulated genes responsible for phosphorus stress in wheat (*Triticum aestivum*) is as follows:

2.1 Data mining of differentially expressed genes related to phosphorus stress response in wheat

Transcriptome profiling data comprising 798 down-response genes was taken into account for dissecting the complex system transcriptome network (Kaur *et al.,* 2021) [6]. To address transcriptomic changes during phosphorus stress, RNA sequencing analysis in response to phosphorus deficiency identified 2983 and 802 genes that were downregulated and upregulated, respectively, and were stored in supplementary tables by Kaur *et al.,* 2021 [6]. Following this, 2983 down-regulated genes were compared with a list of phosphorus use efficiency genes (Kaur *et al.,* 2021; Ray *et al.,* 2020) [6, 16] that was published and contains both up-regulated and down-regulated genes. In common, both datasets share 798 down-regulated genes, and these were utilized for network analysis. In the present study, we have selected a total of 798 differentially expressed genes that were downregulated to cope with low phosphorus. These were utilized for a system biology approach with the aim of characterizing co-expression modules responsive to phosphorus deficiency. To avoid the complexity arising due to the large number of genes (3785 differentially expressed phosphorus stress-responsive genes), we have focused only on the 798 down-regulated genes in phosphorus stress in the recent study. The differentially expressed genes (DEG's) retrieved from the seedling stage transcriptome datasets were utilized to construct a phosphorus stress network using the STRING database (Version 11.5) (Szklarczyk *et al.,* 2015) [22]. In the STRING Database, gene IDs of the seedling stage differentially expressed downregulated genes was submitted. To retrieve the network containing genes with stronger

interactions, a higher confidence level (0.7) was set. The resulting interaction network of the STRING database was utilized to narrow down the potential genes in Cytoscape (Version 3.9.1) (Shannon *et al.*, 2003) [19].

2.2 Construction of Clusters/Sub-networks from the parent network

The network generated from the string database was imported into Cytoscape in order to construct a network depicting the genes that are suppressed under phosphorus stress. Genes (nodes) that lacked connections in the network were chosen to be concealed. The confidence threshold of the Cytoscape network was established at 0.7, aiming to pinpoint highly interconnected nodes (genes with the most edges) within the network. To detect comprehensively interacting sub-networks or clusters of genes, the MCODE plugin in Cytoscape was employed, following the methodology introduced by Bader and Hogue in 2003. The parameter for the degree cut-off was set at 2 to identify the most densely linked sub-networks or clusters.

2.3 Identification of Hub-genes and Bottle-neck genes in relative to down regulation under phosphorus stress

Among the developed sub-networks and clusters, a solitary sub-network (Cluster scoring 20.8) comprising 26 nodes and 260 edge connections was chosen to find hub genes. Within this sub-network, a total of 14 hub genes were recognised, characterized by a minimum of 20 edge connections and a maximum of 25 edge connections. The investigation aimed to uncover which genes exhibit heightened expression during the process of down regulation under phosphorus stress conditions. Cytohubba (Cytoscape plugin application) (Chin *et al.*, 2014) [4] was utilized for the identification of the top 10 genes on the basis of degree and the top 10 bottleneck genes. The top 10 degree-basis genes consist of 10 hub genes, and the top 10 bottleneck genes consist only of 3 hub genes in their network, respectively.

2.4 Gene Ontologies and KEGG enrichment analysis of modules/sub-networks

In order to assess the functional classification of network genes and their participation in diverse metabolic pathways, we undertook a string functional enrichment annotation table provided within Cytoscape. This analysis encompassed gene ontology (GO) evaluation, encompassing cellular components, biological processes, molecular function, and the involvement of the genes in the network. Additionally, we conducted KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis to gain insights into the roles of both hub genes and bottleneck genes in KEGG pathways. A bar graph of gene ontologies, bubble enrichment of KEGG pathways, and multiple bubble graphs (gene ontologies and KEGG pathways) were created using SRPlot software.

3. Results

3.1 Network construction and identification of candidate genes

The entire workflow identified candidate/hub genes central to phosphorus stress tolerance in wheat. We retrieved a total of 798 DEGs pertaining to phosphorus stress responses specific to down regulation and used them for the construction of stress-wise networks. The network constructed had 208 genes (Figure 1).

A total of 3 sub-networks or modules were generated using MCODE for the 208 genes present in the Cytoscape network (Figure 2). The three sub-networks have a cut-off value of 2. The top sub-network or module, considered in terms of interaction density, had a total of 26 genes (Cluster score 20.8) (Figure 3). The smallest module has 4 nodes and 5 edges, while the largest module contains 26 nodes and 260 edges. The 26 candidate genes were analysed and a total of 14 hub-genes were identified with a maximum edge connectivity of 25 and a minimum connectivity of 20 edges. The 14 hub genes, Traes_6DS_8D580A93D.2, Traes_2DS_5814764C5.1, Traes_2DL_20DA8D2D5.1, Traes_5BL_29847C42C.1, Traes_4DS_CC9F9317E.2, Traes_7AS_DC51A1D61.1, Traes_2AL_14B62F6C1.1, Traes_2AL_E7B360A43.1, Traes_2BL_5D64E8C87.1, Traes_2DS_46C9F8F5F.1, Traes_2BS_F698CECA3.2, Traes_6DL_1CCB8CC4B.1, Traes_3B_274BA435E.1, and Traes_4AL_C3729F680.2 were found to be differentially expressed under phosphorus stress (Figure 3) (Table 1). On further analysis of the network of 208 genes in Cytoscape, a total of the top 10 bottleneck genes were identified, and it contains 3 genes out of 10 genes that were also categorized as hub genes (Figure 4). The top 10 bottle-neck genes are Traes_2BL_DD9C6B84.1, Traes_2DS_2CCCA54C1.1, Traes_5DL_F30005957.1, Traes_5DL_43046228D.1, Traes_7DL_15ACBO6B8.1, Traes_4AL_C3729F680.2, Traes_2BL_5D64E8C87.1, Traes_4DL_CB8E1061C.1, Traes_2BS_DDCEEE1DE.1 and Traes_4DS_CC9F9317E.2 and these three hub-genes Traes_2BL_5D64E8C87.1, Traes_4AL_C3729F680.2 and Traes_4DS_CC9F9317E.2 are bottle neck genes (Table 2). On the basis of degree, the top 10 genes were found from the network of 208 genes, and those top 10 genes were hub genes themselves. They are Traes_2AL_14B62F6C1.1, Traes_2AL_E7B360A43.1, Traes_2BL_5D64E8C87.1, Traes_2DL_20DA8D2D5.1, Traes_2DS_46C9F8F5F.1, Traes_3B_274BA435E.1, Traes_4AL_C3729F680.2, Traes_4DS_CC9F9317E.2, Traes_6DS_8D580A93D.2 and Traes_7AS_DC51A1D61.1 (Figure 5) (Table 3). From the bottleneck genes, on the basis of their rank, gene Traes_2BL_5D64E8C87.1 is of high affinity with a rank score of 54 and low affinity gene Traes_4DL_CB8E1061C.1 with a rank score of 6. On the comparison of genes on the basis of degree, the high affinity gene was Traes_2BL_5D64E8C87.1 with a degree score of 36 and the low affinity gene was Traes_2DS_46C9F8F5F.1 with a degree score of 24.

3.2 KEGG Enrichment and GO (Gene ontology) analysis

Gene Ontology (GO) and KEGG pathway analysis of 14 hub genes through string enrichment functional annotation in Cytoscape revealed that most of the hub genes were involved in photosynthesis and related terms to the photosynthesis process. The GO biological process terms related to photosynthesis (GO: 0015979), cellular metabolic process (GO: 0044237) and cellular process (GO: 0009987) were the most represented terms and enriched with all 14 hub-genes related to phosphorus stress. Apart from GO biological terms, in reference to GO molecular function, ion binding (GO: 0043167) and chlorophyll binding (GO: 0016168) consist of 7 and 5 hub genes, respectively. Most of the hub genes were enriched in GO cellular terms, and they are highest compared to the other two GO terms. GO cellular terms such as Cellular anatomical entity (GO: 0110165), Plastid (GO: 0009536),

Chloroplast (GO: 0009507), Cytoplasm (GO: 0005737), and Intracellular (GO: 0005622) are enriched with all 14 hub-genes.

We assigned 14 hub-genes to 5 KEGG pathways (Figure 6) (Table 4). KEGG pathways include photosynthesis-antenna proteins (taes00196), metabolic pathways (taes01100), carbon fixation in photosynthetic organisms (taes00710), photosynthesis (taes00195), and carbon metabolism (taes01200) (Figure 7. a–d). A bar plot of gene ontologies, a bubble enrichment of KEGG pathways, and a multiple group bubble plot (which includes gene ontologies and KEGG pathways) were constructed using the parameters needed through SRPlot software (Figure 8. a–c).

4. Discussion

For sustainable crop development to be promoted, to reduce the use of a scarce resource, and to address environmental concerns, phosphorus must be used more effectively. Enhancing cultivar phosphorus utilization efficiency is seen as a key factor in determining future crop production strategies since it produces plentiful yields with minimal fertilizer use, especially in low-input environments (Vandamme *et al.*, 2016) [24]. Phosphorus use efficiency constitutes a multifaceted agricultural characteristic, encompassing various interconnected stages of phosphorus metabolism and transfer that collaborate with advantageous root and rhizosphere attributes, leading to an enhancement in phosphorus acquisition efficiency (PAE) (Wang *et al.*, 2019) [26]. The complexity of plant PUE arises from its intricate nature, encompassing phosphorus detection, absorption, movement, incorporation, and redistribution. This process is governed by a combination of interlinked genetic factors and environmental variables (Soumya *et al.*, 2021) [21]. The result of the interaction between phosphorus uptake efficiency (PUpE) and phosphorus utilization efficiency (PUtE) is phosphorus use efficiency (PUE). PUpE refers to the root system's ability to take up inorganic phosphate (Pi) from the soil. PUpE improvements are mainly linked to lengthening of the overall root structure, a decrease in primary root growth rate, an increase in the root-to-shoot ratio, augmentation of the root lateral surface, a decrease in axial root growth, as well as lengthening of the root hairs and increased root thickness (Uygur *et al.*, 2018) [23].

In this study, we utilized a total of 798 differentially phosphorus-downregulated expressed genes with their gene identities from scientific journals that were expressed under phosphorus stress to avoid the complexity of the network arising from the large number of genes, and using these genes, a co-expression network was created, displaying an interesting distribution of nodes and edges through Cytoscape. We expected to find major hub genes that were expressed in phosphorus down regulation and their roles in photosynthesis, photosynthesis-related organs, carbon metabolism, and its fixation. Though the 798 differentially down-regulated expressed genes identified for the seedling stage successfully narrowed down to 208 genes, which were analysed for clusters or modules through the MCODE cluster (plug in the Cytoscape app). There were a total of 3 modules or sub-networks, out of which one module was considered, which has a vast network and consists of 26 nodes as genes and 260 edges. On further analysis, 14 genes were considered hub-genes, which were selected based on the concept of their connectivity (number of edges).

GO and KEGG enrichment analysis revealed that the maximum number of hub-genes were involved in the cellular anatomical entity (GO: 0110165), plasmid (GO: 0009536), chloroplast (GO: 0009507), cytoplasm (GO: 0005737), and intracellular (GO: 0005622), in the biological process of photosynthesis (GO: 0015979), the cellular metabolic process (GO: 0044237), and the cellular process (GO: 0009987), and in molecular function as ion binding (GO: 0043167) and chlorophyll binding (GO: 0016168). With respect to KEGG pathway analysis, the maximum hub genes have their roles in photosynthesis: antenna proteins (taes00196), metabolic pathways (taes01100), carbon fixation in photosynthetic organisms (taes00710), photosynthesis (taes00195), and carbon metabolism (taes01200). Maximum number of hub-genes involved in photosynthesis (KEGG pathway) and minimum in carbon fixation in photosynthetic organisms (KEGG pathway) In conclusion, our study identifies key genes (down-regulated) that were expressed under phosphorus stress to improve crop phosphorus use efficiency in phosphorus-deficient soil environments. These genes play a significant role in photosynthesis, which is a major process that plants use to produce food for survival and to produce more yield and biomass. Additionally, investigations or research can be done to improve phosphorus uptake in wheat grown under various abiotic and nutrient stress conditions.

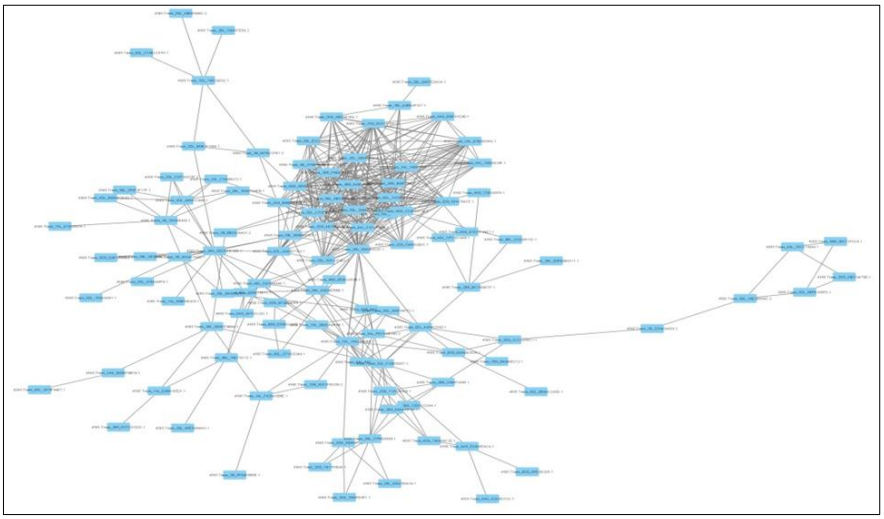


Fig 1: Cytoscape network of 798 differentially expressed down regulated genes in phosphorus stress.

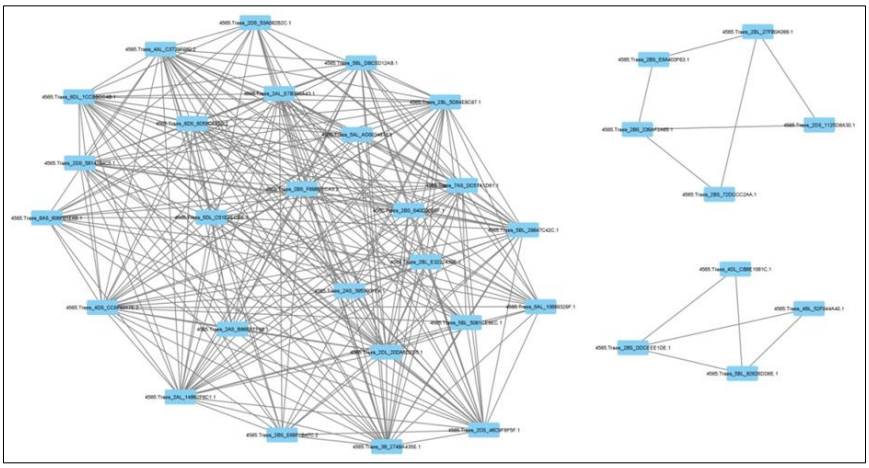


Fig 2: 3 Sub-networks/modules generated using MCODE Cluster

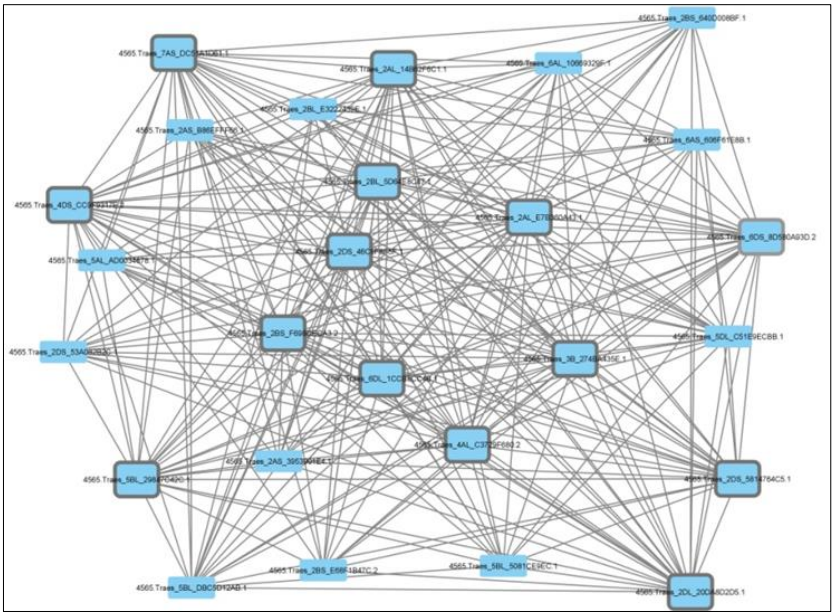


Fig 3: Topmost sub-network/module consists of 26 genes and 206 edges. Highlighted nodes indicates hub genes in the sub-network (14 hub genes)

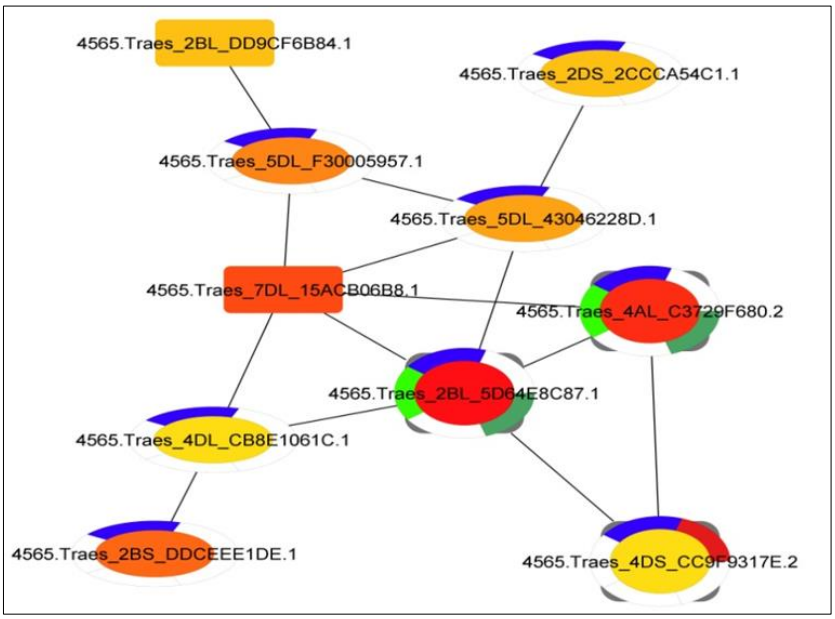


Fig 4: Top 10 bottle neck genes from Cytoscape network with involvement in KEGG pathway indications. Violet- Metabolic pathways, Parrot green- Carbon fixation in photosynthetic organisms, Dark green- Carbon metabolism, Brown- Photosynthesis.

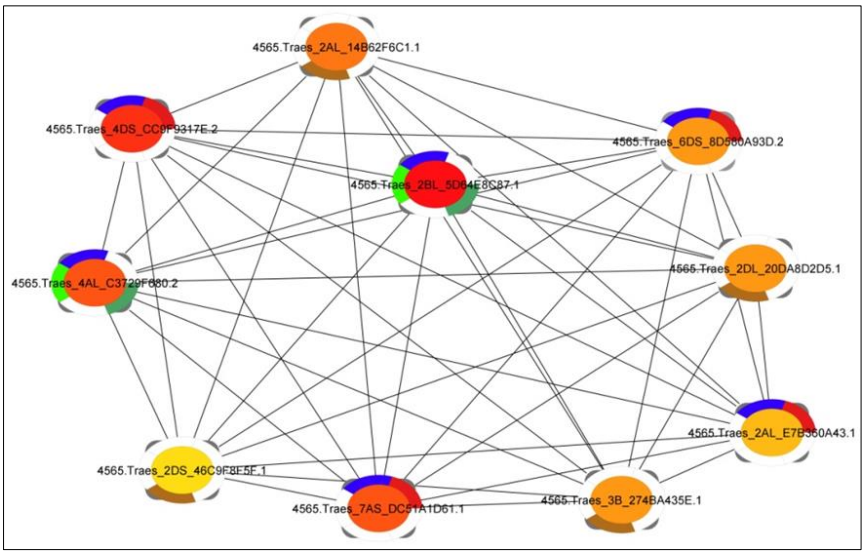


Fig 5: Top 10 genes from network on basis of degree with involvement in KEGG pathway. Violet- Metabolic pathways, Parrot green- Carbon fixation in photosynthetic organisms, Dark green- Carbon metabolism, Brown- Photosynthesis.

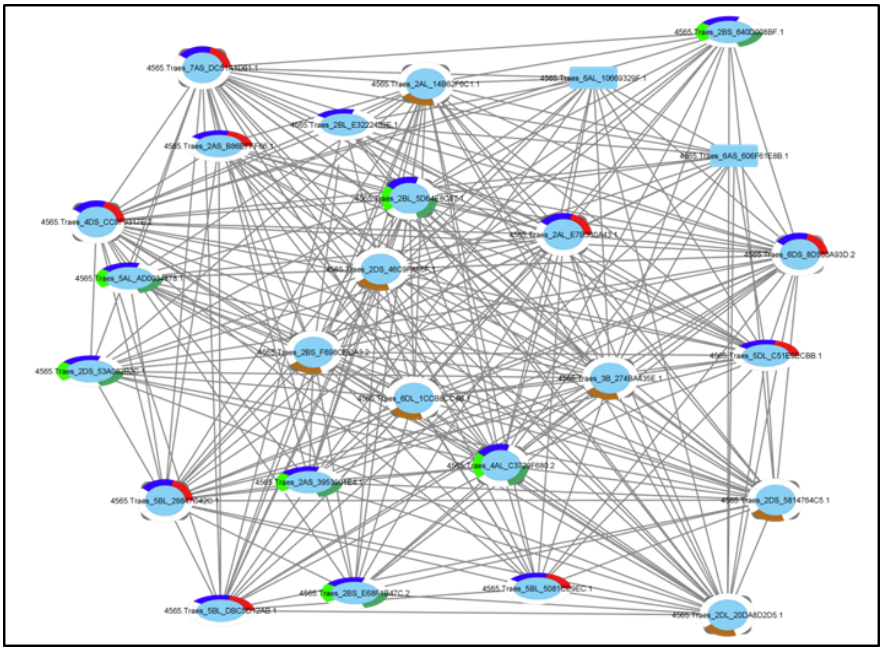
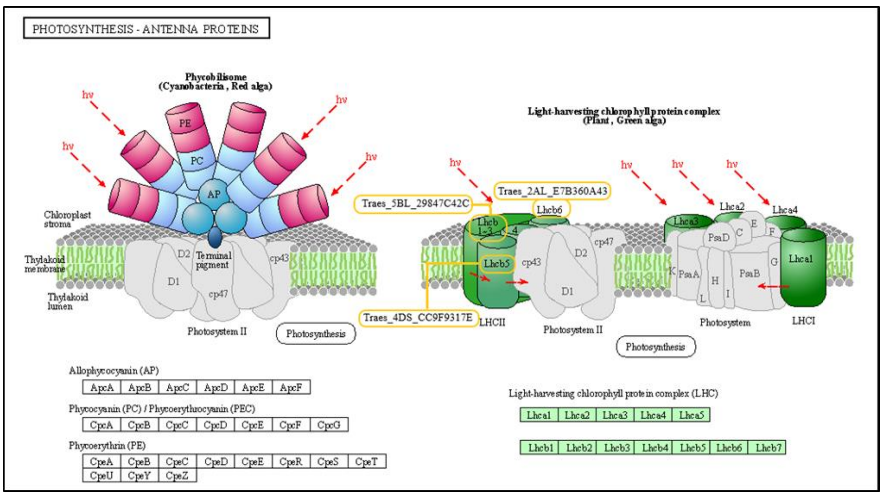
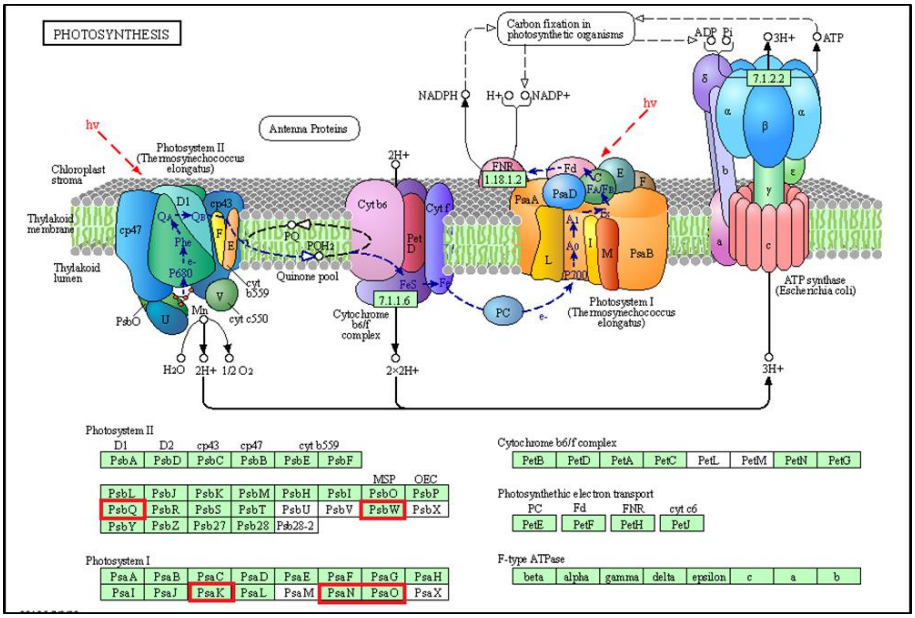
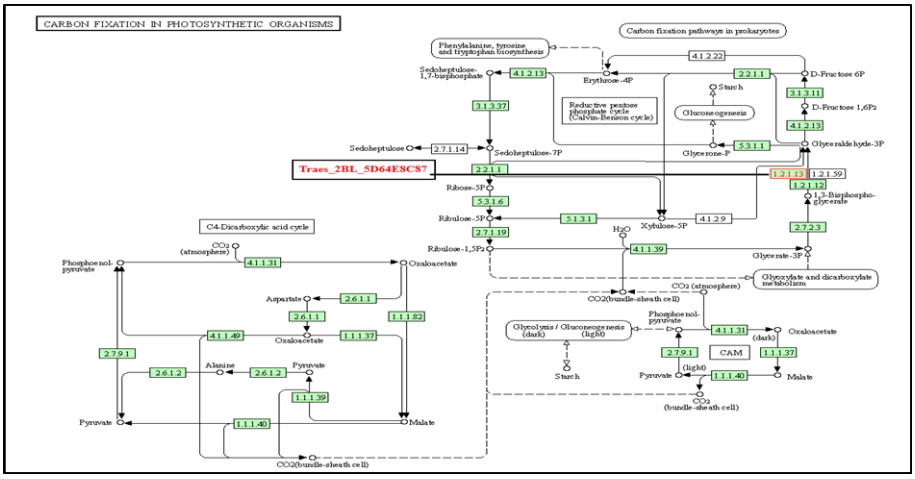


Fig 6: 14 Hub-genes (with dark background) with KEGG pathway indications. Violet- Metabolic pathways, Parrot green- Carbon fixation in photosynthetic organisms, Dark green- Carbon metabolism, Brown- Photosynthesis and Red- Photosynthesis- antenna protein.

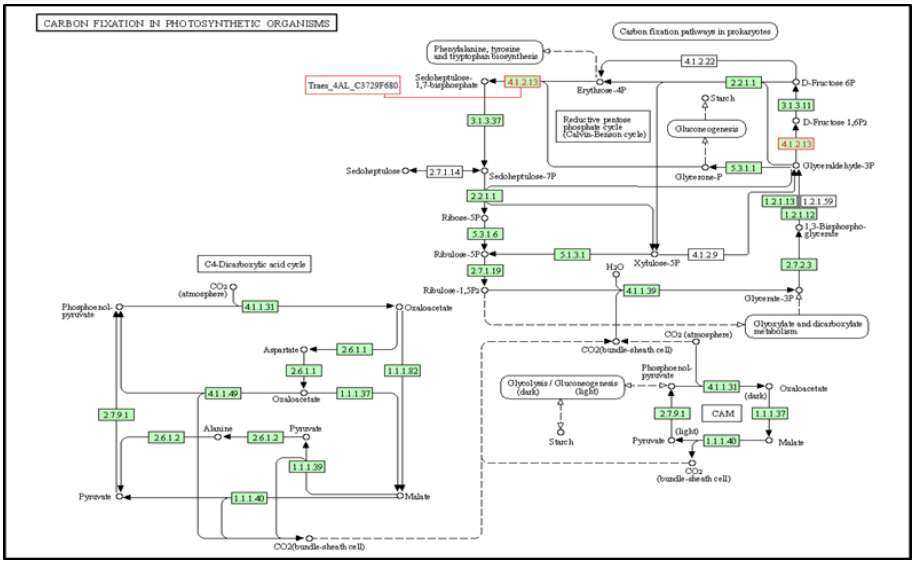




A

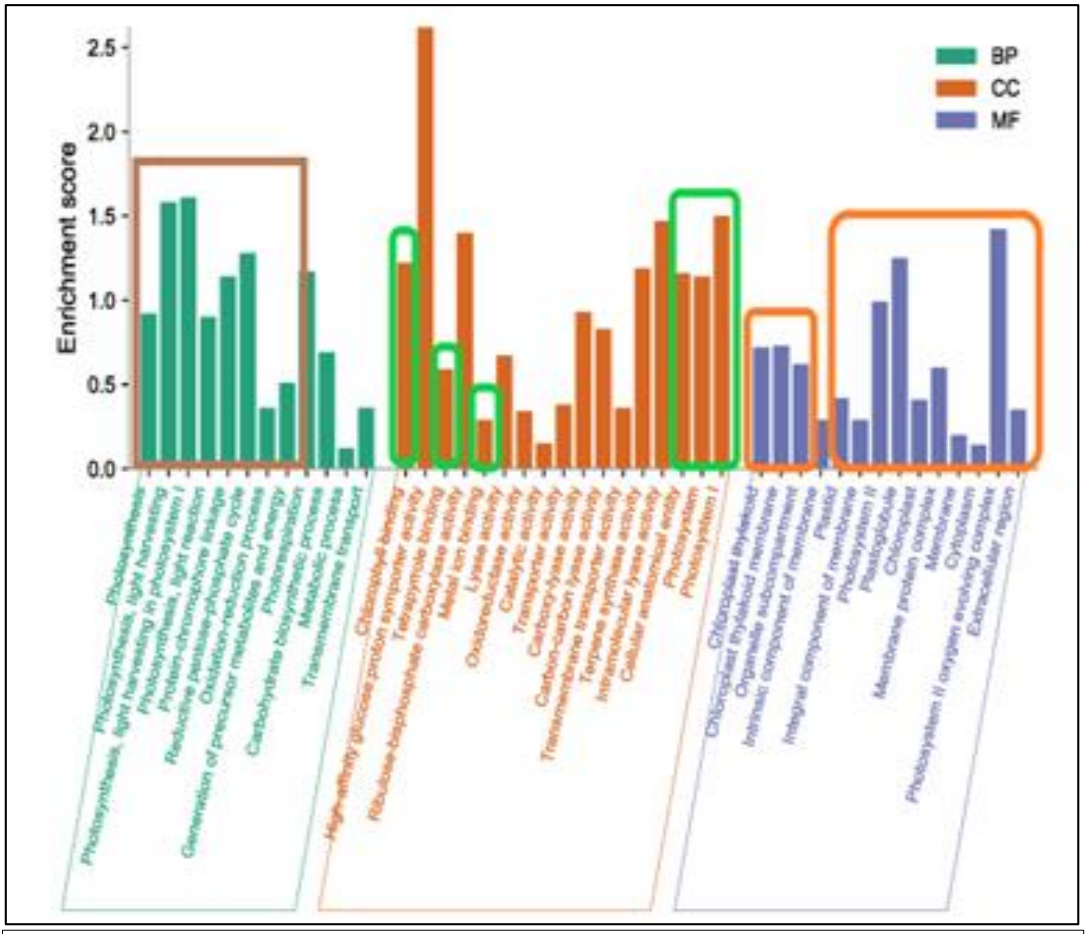


B



C

Fig 7: (a) KEGG pathway showing genes involved in Photosynthesis- antenna proteins. (b) Genes involved in photosynthesis process (highlighted with red). (c), (d) Genes involved in Carbon fixation in photosynthetic organisms (Red colour labelled).


Enrichment score
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2.0
1.5
1.0
0.5
0.0
BP
CC
MF
Photosynthesis
Photosynthesis, light harvesting
Photosynthesis, light harvesting in photosystem I
Photosynthesis, light reaction
Protein-chromophore linkage
Reductive pentose-phosphate cycle
Oxidation-reduction process
Generation of precursor metabolites and energy
Photorespiration
Carbohydrate biosynthetic process
Metabolic process
Transmembrane transport
Chlorophyll binding
Tetrapyrrole binding
Ribulose-bisphosphate carboxylase activity
Metal ion binding
Lyase activity
Oxidoreductase activity
Catalytic activity
Transporter activity
Carbon-carbon lyase activity
Transmembrane transporter activity
Terpene synthase activity
Intramolecular lyase activity
Cellular anatomical entity
Photosystem
Photosystem I
Chloroplast thylakoid membrane
Organelle subcompartment
Intrinsic component of membrane
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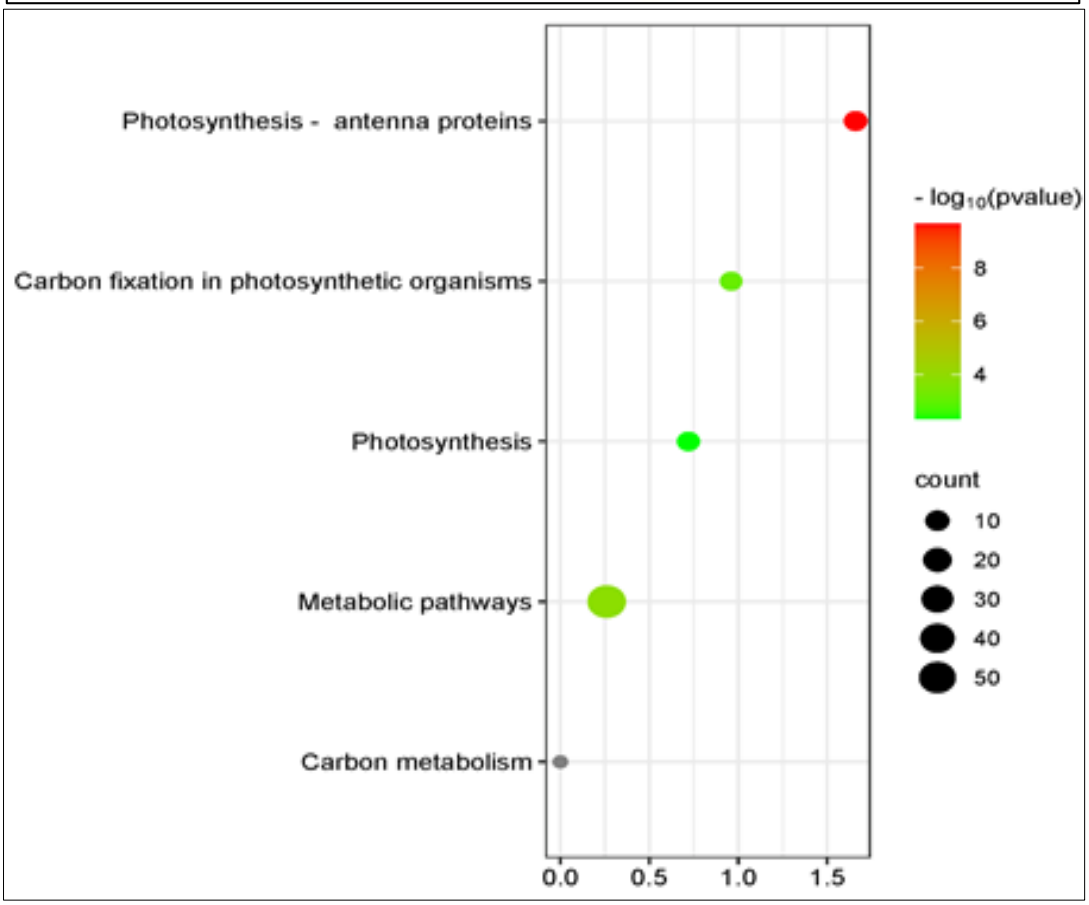
Membrane protein complex
Membrane
Cytoplasm
Photosystem II oxygen evolving complex
Extracellular region


Photosynthesis - antenna proteins
Carbon fixation in photosynthetic organisms
Photosynthesis
Metabolic pathways
Carbon metabolism
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0.5
1.0
1.5
- log10(pvalue)
8
6
4
count
10
20
30
40
50

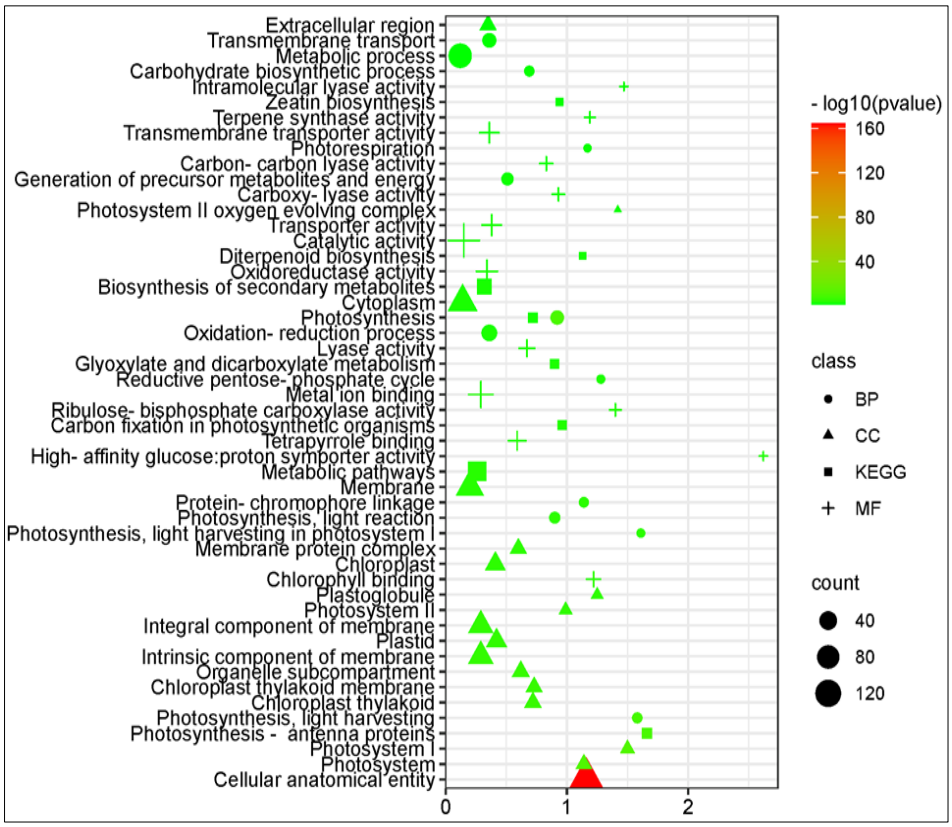

Fig 8: (a) Bar plot of Gene Ontologies highlighted portion indicates hub genes were involved in following gene ontologies. (b) Bubble enrichment plot of KEGG pathways. (c) Multiple group bubble plot involves gene ontologies (Molecular Function, Cellular component and Biological process) and KEGG pathways.

Table 1: List of Hub-genes obtained from clustered network with accession number and their locus identity

Gene Id	Description	Accession No.	Locus Id
Traes_2AL_14B62F6C1.1	Photosystem I reaction center subunit psaK, chloroplastic-like	A0A3B6AYC0	LOC123188752
Traes_2AL_E7B360A43.1	Chlorophyll a-b binding protein CP24 10B, chloroplastic-like	W5AY52	LOC123189406
Traes_2BL_5D64E8C87.1	Glyceraldehyde-3-phosphate dehydrogenase A, chloroplastic	A0A1D5TTT2	LOC123045658
Traes_2BS_F698CECA3.2	Oxygen-evolving enhancer protein 3, chloroplastic	A0A3B6C475	LOC100049048
Traes_2DL_20DA8D2D5.1	Photosystem I subunit O-like	W5BS13	LOC123053251
Traes_2DS_46C9F8F5F.1	Oxygen-evolving enhancer protein 3, Chloroplastic-like	W5C4P1	LOC123052491
Traes_2DS_5814764C5.1	Photosystem I reaction center subunit N, Chloroplastic	W5C572	LOC123051617
Traes_3B_274BA435E.1	Photosystem II reaction center W protein, psbW	A0A077S4A0	LOC123070959
Traes_4AL_C3729F680.2	Fructose-bisphosphate aldolase, Chloroplastic-like	W5DTC2	LOC123086572
Traes_4DS_CC9F9317E.1			
Traes_5BL_29847C42C.1	Chlorophyll a-b binding protein, chloroplastic	W5F8Z5	LOC123112836
Traes_6DL_1CCB8CC4B.1	Photosystem II 10 kDa polypeptide, chloroplastic-like	A0A3B6QMM8	LOC123146030
Traes_6DS_8D580A93D.2	Chlorophyll a-b binding protein 8, chloroplastic-like	W5GFA4	LOC123144198
Traes_7AS_DC51A1D61.1	Chlorophyll a-b binding protein 1B-21, chloroplastic	C1K5B8	LOC100415887

Table 2: Description of top 10 Bottle Neck genes from the network of differentially expressed downregulated genes in phosphorus stress.

Gene Id	Description	Accession no.	Locus id	KEGG pathway
Traes_2BL_5D64E8C87.1	Glyceraldehyde-3-phosphate dehydrogenase A, chloroplastic	A0A1D5TTT2	LOC123045658	Metabolic pathway (taes01100) Carbon fixation in photosynthetic organisms (taes00710) Carbon metabolism (taes01200)
Traes_2BL_DD9CF6B84.1	Endonuclease 1	A0A3B6CFB8	LOC123042524	----
Traes_2BS_DDCEEE1DE.1	Probable 5'-adenylylsulfate reductase 1, chloroplastic	A0A3B6C812	LOC123044707	Metabolic pathway (taes01100)
Traes_2DS_2CCCA54C1.1	Peroxidase	Q05855	LOC123184735	Metabolic pathway (taes01100)
Traes_4AL_C3729F680.2	Fructose-bisphosphate aldolase, chloroplastic-like	W5DTC2	LOC123086572	Metabolic pathway (taes01100) Carbon fixation in photosynthetic organisms (taes00710) Carbon metabolism (taes01200)
Traes_4DL_CB8E1061C.1	Glutamine synthetase cytosolic isozyme 1-2-like	Q6RUJ1	LOC123098202	Metabolic pathway (taes01100)
Traes_4DS_CC9F9317E.2	Chlorophyll a-b binding protein CP26, chloroplastic-like	A0A096URP1	LOC123096214	Metabolic pathway (taes01100) Photosynthesis- antenna protein (taes00196)

Traes_5DL_43046228D.2				
Traes_5DL_F30005957.1	Alcohol dehydrogenase 2	A0A3B6MPR3	LOC123121343	Metabolic pathway (taes01100)
Traes_7DL_15ACB06B8.1	Aldehyde dehydrogenase family 2 member C4-like	A0A3B6TMJ5	LOC123168948	---

Table 3: Top 10 Hub-genes on basis of Degree through Cytohubba

Gene Id	Description	Accession No.	Locus Id
Traes_2AL_14B62F6C1.1	Photosystem I reaction center subunit psaK, chloroplastic-like	A0A3B6AYC0	LOC123188752
Traes_2AL_E7B360A43.1	Chlorophyll a-b binding protein CP24 10B, chloroplastic-like	W5AY52	LOC123189406
Traes_2BL_5D64E8C87.1	Glyceraldehyde-3-phosphate dehydrogenase A, chloroplastic	A0A1D5TTT2	LOC123045658
Traes_2DL_20DA8D2D5.1	Photosystem I subunit O-like	W5BS13	LOC123053251
Traes_3B_274BA435E.1	Photosystem II reaction center W protein, psbW	A0A077S4A0	LOC123070959
Traes_4AL_C3729F680.2	Fructose-bisphosphate aldolase, Chloroplastic-like	W5DTC2	LOC123086572
Traes_4DS_CC9F9317E.1			
Traes_5BL_29847C42C.1	Chlorophyll a-b binding protein, chloroplastic	W5F8Z5	LOC123112836
Traes_6DS_8D580A93D.2	Chlorophyll a-b binding protein 8, chloroplastic-like	W5GFA4	LOC123144198
Traes_7AS_DC51A1D61.1	Chlorophyll a-b binding protein 1B-21, chloroplastic	C1K5B8	LOC100415887

Table 4: Hub genes involved in different KEGG pathways

GENE ID	KEGG Pathway	KEGG Description
Traes_2AL_14B62F6C1.1	taes00195	Photosystem I reaction centre subunit, psaK
Traes_2AL_E7B360A43.1	taes00196 taes01100	Chlorophyll a-b binding protein, chloroplastic (LHCB6) Metabolic Pathways
Traes_2BL_5D64E8C87.1	taes01200 taes00710	Carbon metabolism Carbon fixation in photosynthetic organisms (EC1.2.1.13)
Traes_2BS_F698CECA3.2	taes00195	Photosynthesis (Oxygen evolving enhancer protein 3, psbQ)
Traes_2DL_20DA8D2D5.1	taes00195	Photosynthesis (Photosystem I subunit O, psaO)
Traes_2DS_46C9F8F5F.1	taes00195	Photosynthesis (Oxygen evolving enhancer protein 3-2, psbQ)
Traes_2DS_5814764C5.1	taes00195	Photosynthesis (Photosystem I reaction centre subunit N, psaN)
Traes_3B_274BA435E.1	taes00195	Photosynthesis (Photosystem II reaction centre W protein, psbW)
Traes_4AL_C3729F680.2	taes01200	Carbon metabolism (Fructose bi phosphate aldolase class I)
Traes_4DS_CC9F9317E.1	taes01100	Metabolic Pathways (Light harvesting complex II, chlorophyll a b binding protein 5, lhcb5)
Traes_5BL_29847C42C.1	taes00196, taes01100	Photosynthesis – antenna proteins (Light harvesting complex II, chlorophyll a b binding protein 2, lhcb2) Metabolic pathways
Traes_6DL_1CCB8CC4B.1	NA	NA
Traes_6DS_8D580A93D.2	taes00196 taes01100	Photosynthesis- antenna proteins (Chlorophyll a b binding protein 8) Metabolic Pathways
Traes_7AS_DC51A1D61.1	taes00196 taes01100	Photosynthesis antenna proteins Metabolic Pathways

5. Conclusion

The current study has identified a set of candidate genes which can provide basic tolerant machinery to crop plants for challenging against nutrient stresses at least at seedling stage. These 14 hub-genes can serve as potential source for increasing different metabolic processes in wheat crops which can make increments in the yield and biomass production and by enhancing expression of genes plant can be more tolerant to phosphorus stress conditions.

6. References

1. Abbas H, Naeem MK, Rubab M, Widemann E, Uzair M, Zahra N, *et al*. Role of Wheat Phosphorus Starvation Tolerance 1 Genes in Phosphorus Acquisition and Root Architecture. Genes. 2022;13:487.
2. Appels R, Eversole K, Feuillet C, Keller B, Rogers J, Stein N, *et al*. Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science; c2018. p. 361.
3. Bader GD, Hogue CW. An automated method for finding molecular complexes in large protein interaction networks. BMC Bioinform. 2003;4(1):2.
4. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY, *et al*. Cytohubba: Identifying hub objects and sub-networks from complex interactome. BMC Syst Biol. 2014;8:2018.
5. Dhillon J, Torres G, Driver E, Figueiredo B, Raun WR. World phosphorus use efficiency in cereal crops. Agronomy Journal; c2017.
6. Kaur G, Shukla V, Meena V, Kumar A, Tyagi D, Singh J, *et al*. Physiological and molecular responses to combinatorial iron and phosphate deficiencies in hexaploid wheat seedlings. Genomics, 2021, 113(6).
7. Juan LX, Jin GC, Jing LW, Wei DW, Miao Z, Ying MC, *et al*. Expression Pattern Analysis of Zinc Finger Protein Genes in Wheat (*Triticum aestivum* L.) Under Phosphorus Deprivation. Journal of Integrative Agriculture. 2014;13(8):1621-1633.
8. Krasilnikov P, Taboada MA, Amanullah. Fertilizer Use, Soil Health and Agricultural Sustainability. Agriculture 2022;12;462.
9. Kothari D, Pargaien N, Tewari LM, Dikshit HK, Mishra GP, Aski MS, *et al*. Genetic Variation for Traits Related to Phosphorus Use Efficiency in Vigna Species. Agronomy. 2023;13:305.
10. McDonald G, Bovill W, Taylor J, Wheeler R. Responses to phosphorus among wheat genotypes. Crop Pasture Science. 2015;66:430–444.
11. Ming F, Zheng X, Mi G, He P, Zhu L. Identification of

quantitative trait loci affecting tolerance to low phosphorus in rice (*Oryza Sativa* L.). Chin. Sci. Bull. 2000;45:520–525.
12. Ozlu E, Kumar S. Response of Soil Organic Carbon, pH, Electrical Conductivity, and Water Stable Aggregates to Long-Term Annual Manure and Inorganic Fertilizer. Soil Sci. Soc. Am. J. 2018:82:1243.
13. Pahalvi HN, Rafiya L, Rashid S, Nisar B, Kamili AN. Chemical Fertilizers and Their Impact on Soil Health in Micro biota and Biofertilizers. Springer: Cham, Switzerland. 2021;2:1–20.
14. Penn CJ, Camberato J. A critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. Agriculture. 2019;9:120.
15. Plaimas K, Eils R, Konig R. Identifying essential genes in bacterial metabolic networks with machine learning methods. Plant and Soil. 2010;439:91–112.
16. Ray P, Guo Y, Chi MH, Krom N, Malay C, Saha KD. *Serendipita bescii* promotes winter wheat growth and modulates the host root transcriptome under phosphorus and nitrogen starvation. Applied Microbiology International. 2020;23(4):1876-1888.
17. Rezakhani L, Motesharezadeh B, Tehrani MM, Etesami H, Hosseini HM. Phosphate–solubilizing bacteria and silicon synergistically augment phosphorus (P) uptake by wheat (*Triticum aestivum* L.) plant fertilized with soluble or insoluble P source. Ecotoxicology and Environmental Safety. 2019;173:504–513.
18. Shaik NA, Hakeem KR, Banaganapalli B, Elango R. Essentials of Bioinformatics, Understanding Bioinformatics: Genes to Proteins Biological Networks Tools, Methods, and Analysis; c2019. p. 255–286.
19. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, *et al*. Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Research. 2003;13(11):2498–2504.
20. Sharma I, Tyagi BS, Singh G, Venkatesh K, Gupta OP. Enhancing wheat production- a global perspective. Indian J Agril Sci. 2015;85:3–13.
21. Soumya PR, Sharma S, Meena MK, Pandey R. Response of diverse bread wheat genotypes in terms of root architectural traits at seedling stage in response to low phosphorus stress. Plant Physiology Report. 2021;26:152–161.
22. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, *et al*. STRING v10: Protein–protein interaction networks, integrated over the tree of life. Nucleic Acids Research. 2015;43(D1):D447–D452.
23. Uygur V, Mustafa S. The effect of phosphorus application on nutrient uptake and translocation in wheat cultivars. Intern. J Agric. Forest. Life Science 2018;2:171–179.
24. Vandamme E, Rose T, Saito K, Jeong K, Wissuwa M**.** Integration of P acquisition efficiency, P utilization efficiency and low grain P concentrations into P-efficient rice genotypes for specific target environments. Nutrient Cycling in Agroecosystems. 2014;104:413–427.
25. Venske E, Dos RSS, Busanello C, Gustafson P, de OAC. Bread wheat: A role model for plant domestication and breeding. Hereditas. 2019;156:1–11.
26. Wang J, Qin Q, Pan J, Sun L, Sun Y, Xue Y, *et al*. Transcriptome analysis in roots and leaves of wheat seedlings in response to low-phosphorus stress. Sci Report. 2019;9:19802.
27. Yasmeen H, Yaseen M, Naveed M, Arfan M. Effect of hormones enriched polymer coated fertilizer on growth, yield and phosphorus use efficiency of wheat (*Triticum aestivum* l.) under salinity stress. Pak. J Agri. Sci. 2021;58(3):905-917.