

Research Article

## ***In-silico* Analysis of 2-cysteine peroxiredoxin Genes in *Arabidopsis thaliana* with Possible Role in Carbon Dioxide Fixation through Carbonic Anhydrase regulation**

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**Abstract:** Targeting first neighbors and adding interactive protein to investigate the direct and indirect influence of neighbor genes is an important novel technique used in cancer studies. Weighing neighbor genes based on topological analysis methods for essential protein identification and multi-omics data helps predict significant interactions in such investigations. A similar approach can also be adopted to determine the specific genes in photosynthetic research, especially under stress conditions like low CO<sub>2</sub> and the transformation of plants from C<sub>3</sub> to C<sub>4</sub> photosynthesis. In this study, a computational approach has been used to identify two Genes, i.e., 2-cys peroxiredoxin A (2CPA; *AT3G11630/BAS1*) and 2-cysteine peroxiredoxin B (2CPB; *AT5G06290*) that may regulate Carbonic anhydrase during CO<sub>2</sub> fixation process during photosynthesis in *Arabidopsis thaliana*. We have retrieved almost one hundred genes in the Protein-Protein Interaction (PPI) network of Carbonic anhydrase and identified the hub proteins in the network using Cyto-Hubba and AraNet gene prioritization tools to show significant interactions of essential proteins with Carbonic anhydrase (CA). In the top thirty-four (34) essential proteins, the GeneMania web tool predicted direct interactions among six proteins, i.e. Photosystem I P subunit (CURT1B: *AT2G46820*), Photosystem I subunit 1 (PSAL: *AT4G12800*), Ferredoxin-NADP[+]-oxidoreductase 1 (FNR1: *AT5G66190*), ATPase (F0 complex subunit B/B' (PDE334: *AT4G32260*), Glucose-6-phosphate/phosphate translocator-like protein (TPT; *AT5G46110*) and Photosystem I subunit H2 (PSAH2; *AT1G52230*) that were participating in the same biological process and are found in the chloroplast. Upon addition of interactor protein, BAS1 and 2CPB were also found to have significant interactions with CA based on multi-omics data. GO Biological process enrichment analysis confirmed that six genes are involved in photosynthesis except for BAS1 and 2CPB. These genes may interact with CA and possibly regulate it under high temperature and low CO<sub>2</sub> conditions.

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## 1. Introduction

Photosynthesis is converting electromagnetic energy into chemical energy stored in organic materials. Light supplies this energy and is fixed by chlorophyll. It is a series of chemical energy-producing processes from electromagnetic energy in photosynthetic organisms (Gest, 1993). Photosynthesis occurs differently in different species (Reece et al., 2011), divided into light-dependent and independent reactions. The light-independent reaction refers to the Calvin cycle, fixing carbon dioxide ( $\text{CO}_2$ ) from inorganic to an organic compound. 3-phosphoglycerate (3-PGA) is reduced into glyceraldehyde-3-phosphate (GAP) with the help of NADPH and ATP, which themselves are converted into  $\text{NADP}^+$  and Adenosine Diphosphate (ADP). Ribulose biphosphate (RuBP) is regenerated in the last step, allowing the system to fix more  $\text{CO}_2$  (Raven, 2005). In the light-dependent reaction, pheophytin; a modified form of chlorophyll, absorbs the high-energy electron and passes it to another molecule called quinone, thus resulting in a continuous flow of electrons. This flow of electrons is called the electron transport chain, which causes a reduction of NADP to NADPH. The electron transport chain creates an energy gradient across the chloroplast membrane (Raven, 2005).

Photosynthesis has three types based on the  $\text{CO}_2$  fixation process, i.e., CAM, C3, and C4. Crassulacean acid metabolism (CAM) photosynthesis occurs in two steps, i.e. (1) C4 acid is synthesized with the help of phosphoenol pyruvate carboxylase (PEPC) during night time followed by  $\text{CO}_2$  re-fixation and decarboxylation that is caused by Ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) during day time (Smith, 1992). During C3 photosynthesis, two molecules of phosphoglycerate are formed from  $\text{CO}_2$  through RUBISCO. The RUBISCO enzyme catalysed two distinct reactions, i.e., the formation of phosphoglycerate when oxygen is used as a substrate and the formation of phosphoglycerate when  $\text{CO}_2$  is used as a substrate. Lesser  $\text{CO}_2$  is fixed through an oxygenase reaction known as photorespiration (Ehleringer and Monson, 1993). C4 photosynthesis is a sum of morphological and biochemical modifications in C3 photosynthesis, which improves the photosynthetic environment by increasing  $\text{CO}_2$  around RUBISCO, which reduces its oxygenase activity. In C4 plants, the C3 cycle is restricted to the bundle sheath cells, while mesophyll cells (surrounding the bundle sheath cells) have a more active PEPC enzyme. The C4 pathway is the best solution under high temperature and low  $\text{CO}_2$  atmospheres. C4 photosynthesis controls photorespiration activity which occurs at a higher rate in C3 plants under high temperatures and the environmental conditions that lower  $\text{CO}_2$  concentration around RUBISCO (Ehleringer et al., 1997). C3 plants can store and capture less  $\text{CO}_2$  than C4 plants. So C4 photosynthesis is more productive under high-temperature conditions (Kellogg, 2013).

Carbonic anhydrase catalyses the conversion of CO<sub>2</sub> into HCO<sub>3</sub> in mesophyll cells (Kellogg, 2013). It has two isoforms, i.e., chloroplastic and cytosolic forms, based on their differential actions in different sub-cellular portions (Hatch and Burnell, 1990). The chloroplastic form contributes more in C<sub>3</sub> plants and is predominant in C<sub>4</sub> plants (Hatch and Burnell, 1990).

It is essential to understand the protein-protein interaction networks of any protein. It reveals significant interactions among known proteins in a pathway and helps identify new genes in the same biological pathway. Presently, a lot of research is being conducted on photosynthesis and the behaviours of plants under high stress and low CO<sub>2</sub> conditions. We have adopted a bioinformatic approach to identify new genes that may have a role in regulating CA during carbon dioxide fixation. We have analysed the dense PPI network of Carbonic anhydrase and identified hub genes using computational approaches. The Gene prioritization technique has been used to identify more significant genes among hub proteins and then identify predicted interactions. These interactions share the same biological process and molecular function and are in the same cellular components as Carbonic anhydrase. An additional Interactor protein was added to identify strong indirect interactions among the hub proteins and CA. Through GO biological process enrichment analysis, it has been observed that six prioritized genes and one additional interactor protein show strong predicted direct interactions with CA. In contrast, one of the hub proteins has shown promising indirect interactions with CA. Since six of the above genes are already involved in photosynthesis, the remaining two could be significant in CA regulation under stressed conditions.

## **2. Materials and Methods**

### **2.1. Retrieval of Nucleotide and protein sequences**

The gene (At3g01500) responsible for coding beta Carbonic anhydrase and the protein sequence of beta Carbonic anhydrase were retrieved from TAIR (<https://www.arabidopsis.org>) database and were further subjected to computational tools for annotation.

### **2.2. PPI Network Construction and Analysis**

The protein-protein interaction network of beta Carbonic anhydrase was generated with the help of the STRING database (<http://string-db.org>). STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) is a freely accessible biological database that provides known and predicted Protein-Protein interactions (Mering et al., 2003). The network analysis was performed using Cytoscape, a project of integrated biomolecular interaction networks with expression data and other molecular states in one single place (Shannon et al., 2003).

### 2.3. Identification of Hub proteins

The Hub proteins were identified through Maximal Clique Centrality (MCC) technique for hub proteins analysis provided by Cyto-HUBBA (<http://hub.iis.sinica.edu.tw/cytohubba/>). MCC has the highest precision in identifying essential proteins among all the topological analysis methods for hub protein identification (Chin et al., 2014). The Hub proteins were further prioritized using a probabilistic approach for omics data integration to find more probable significant interactions using an Arabidopsis thaliana-specific computational tool, i.e. AraNet v2 (<https://www.inetbio.org/aranet/>) (Zhai et al., 2016).

### 2.4. Identification and analysis of predicted interactions

The top common essential proteins were looked for interactions that were previously not reported in the literature using GeneMania (<http://genemania.org>) tool to predict the function and interactions of proteins (Franz et al., 2018). The multi-omics data generated the predicted interactions (Lee et al., 2010). All the predicted interactions were then separately screened to look for those proteins involved in the same biological process, molecular function, or localized in the exact cellular location in the presence of an additional interactor gene showing strong interactions with hub proteins CA.

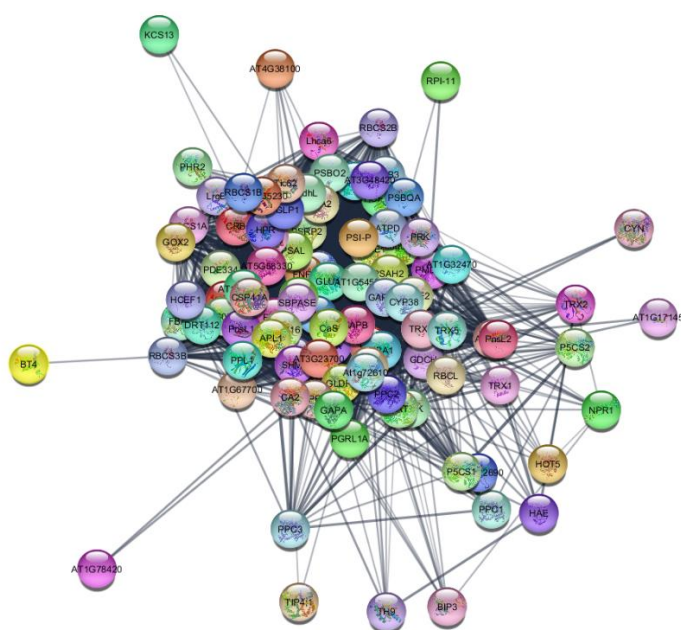
### 2.5. Enrichment Analysis of GO; Biological Process

Gene Ontology (GO) biological process enrichment analysis of the prioritized genes was performed using Database for Annotation Visualization and Integrated Discovery (DAVID's) functional annotation tool. DAVID is an integrated database of Genomic experiments and has a high throughput data-mining environment that mines data and provides large-scale enriched genomic data (Huang et al., 2019).

## 3. Results

### 3.1. PPI Network Construction and Analysis of Carbonic anhydrase

The PPI network of Carbonic anhydrase was constructed using the STRING database. To analyze all the possible significant interacting proteins with Carbonic anhydrase, the minimum nodes were set to 100, and the confidence score was set to 0.4, which infers medium confidence. A dense PPI network of 101 Nodes, 2730 Edges, and 54.1 intermediate nodes degree was obtained (Figure 1). The average local clustering coefficient was 0.865, the expected number of edges was 527, and the PPI enrichment p-value was calculated as  $<1.0e-16$ .



**Figure 1: Beta Carbonic anhydrase mediated PPI network of *Arabidopsis thaliana* generated by STRING database.** The figure shows a dense network of 101 nodes having 2730 edges. The data were retrieved from different sources, e.g., databases, experiments, text mining, gene fusion, and co-occurrence of genes and proteins.

### 3.2. Identification and Prioritization of Significant/Hub Proteins

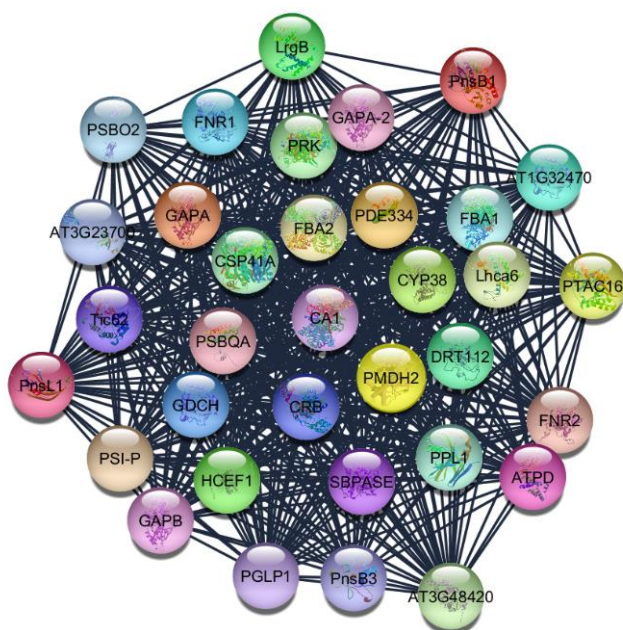
A dense network of 101 proteins was analysed through Cyto-Hubba and 34 hub proteins were identified based on Maximal Clique Centrality (MCC).

**Table 1: Common Hub Proteins between AraNetv2 and Cyto-Hubba CA-mediated PPI network tools based on Maximum Clique Centrality (MCC).**

	Nodename	MCC	S.No	Node name	MCC
1	AT4G25050.2	9.22E+13	18	AT3G14930.2	9.22E+13
2	AT1G19730.1	4727546	19	AT5G19220.1	9.22E+13
3	AT3G23700.1	9.22E+13	20	AT2G40490.1	9.22E+13
4	AT5G46110.4	9.22E+13	21	AT1G52230.1	9.22E+13
5	AT1G65230.1	9.22E+13	22	AT3G52150.1	9.22E+13
6	AT3G18890.1	9.22E+13	23	AT5G23060.1	9.22E+13
7	AT4G38100.1	240	24	AT3G46780.1	9.22E+13
8	AT1G67700.2	9.22E+13	25	AT4G12800.1	9.22E+13
9	AT5G66190.1	9.22E+13	26	AT4G32260.1	9.22E+13
10	AT2G25810.1	2	27	AT5G06290.1	9.22E+13
11	AT2G46820.1	9.22E+13	28	AT2G39800.1	6.54E+12
12	AT3G26060.2	9.22E+13	29	AT5G43750.1	9.22E+13
13	AT5G43940.2	120	30	AT3G55610.1	6.54E+12
14	ATCG00490.1	9.22E+13	31	AT2G03440.1	2
15	AT3G14415.2	9.22E+13	32	AT5G07020.1	9.22E+13

16	AT3G48730.1	9.22E+13	33	AT4G22890.1	9.22E+13
17	AT4G33010.1	9.22E+13	34	AT4G35090.1	9.22E+13

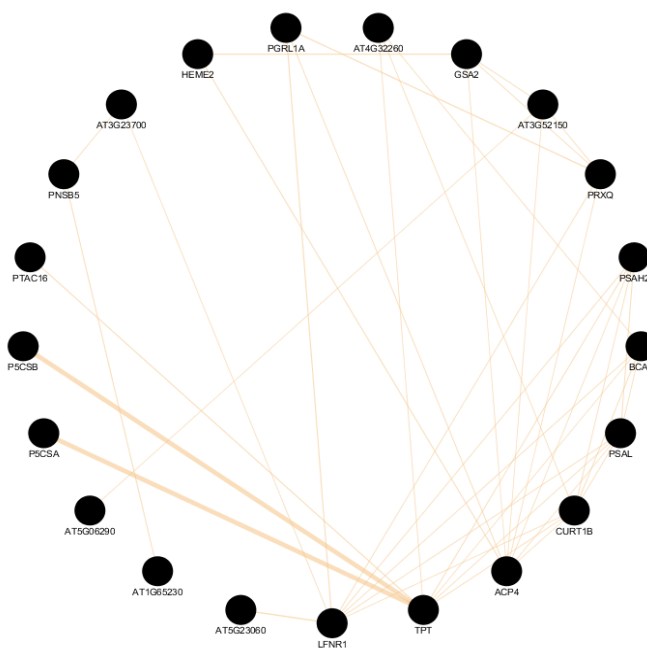
The Hub proteins from the Cyto-Hubba server were prioritized by using AraNettool. Using different algorithms, this Gene Prioritization tool ranks the genes in a tabular list based on their significance. To increase the accuracy and weightage of the identified essential proteins, a comparison was drawn among the top 50 significant proteins from both Cyto-Hubba and AraNet tools. Thirty-four proteins were common between the two selected for further analysis (Figure 2) (Table 1).



**Figure 2:** CA1 mediated interactions network of thirty-four top pick proteins. The proteins were extracted from the network as essential based on Maximum clique centrality (MCC) using Cyto-Hubba. These proteins were also prioritized by the AraNet v2 tool. The figure is generated by the STRING layout filter provided by Cytoscape.

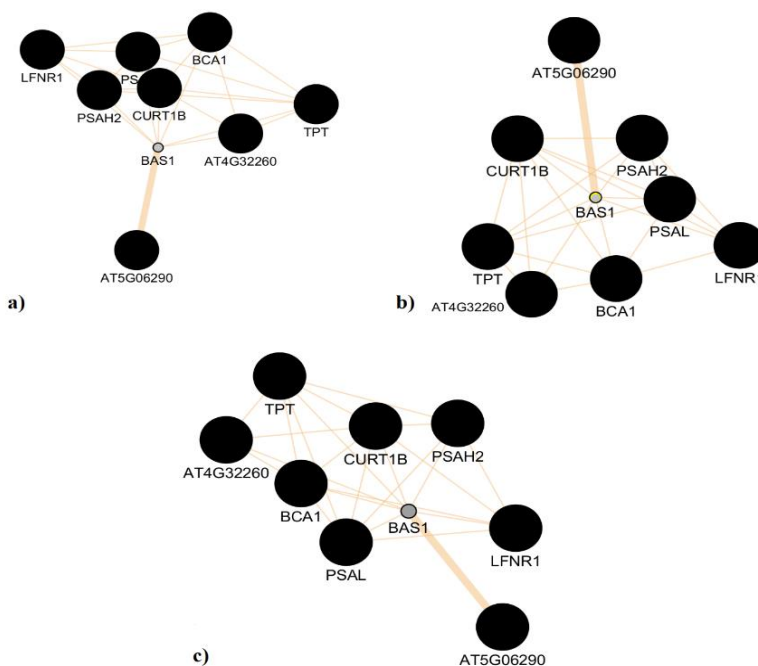
### 3.3. Identification and Analysis of Predicted Interactions

All the thirty-four prioritized hub proteins were subjected to the GeneMania web tool to search for the predicted interactions. Among these proteins, twenty-three proteins interact with each other. The interactions were then predicted separately based on biological processes, molecular functions, and cellular components (Figure 2).



**Figure 3:** GeneMania generated predicted interactions among the top thirty-four essential proteins. The figure shows all the predicted interactions that directly interact with Carbonic anhydrase and regulate it. No neighbour interactor is added in the predicted interactions network of Carbonic anhydrase 1 above. The predicted interactions were generated based on multi-omics data.

Twenty-one proteins of thirty-four hub proteins have predicted solid interactions. In comparison, six proteins (CURT1B, PSAL, FNR1, PDE334, TPT, and PSAH2) share the same molecular function, are involved in the same biological process, and are localized in the same cellular component. These six genes and one additional interactor protein have strong predicted interactions with CA. However, another interactor protein, i.e. 2CPA/BAS1; AT3G11630, a new gene, i.e. 2CPB; At5g06290 (UniprotKB - Q96291), has shown promising interaction with CA1 (Figure 3). Surprisingly this protein is also part of the essential proteins of the CA1 mediated network. These results highlight the possible role of 2CPA and 2CPB protein in carbon fixation by regulating CA during C4 photosynthesis and can be vital during the transformation of plants from C3 to C4 photosynthesis as it can be responsible for up or down-regulation of CA1 that may significantly alter carbon fixation.



**Figure 4:** Predicted interactions of Carbonic anhydrase with hub proteins of its network based on the same (a) Molecular function, (b) Biological process and (c) Cellular function. The figure is generated by the GeneMania plugin in Cytoscape with 2-Cys peroxiredoxin BAS1 (UniprotKB - Q96291) interactor protein to show the predicted interactions among CA1 and other core proteins in the network of CA1 from multi-omics data14.

### 3.4. GO Biological Process Enrichment Analysis of Prioritized Genes

GO enrichment analysis of six prioritized genes was performed by DAVID using the GO Biological Process filter. The results revealed that five out of the six prioritized genes participate in various photosynthetic processes like ATP Synthesis, Proton Transport, photosynthetic acclimation, photosynthetic electron transport chain, and Photosynthetic Light reaction.

**Table 2:** GO Biological Process Enrichment Analysis of the Six Prioritized genes/Proteins by DAVID tool. The table shows the core proteins of the CA1 mediated network and its involvement in the photosynthesis process.

ID	Gene/ Protein Name	Species	GOTERM_BP_DIRECT
AT1G52230	photosystem I subunit H2(PSAH2)	<i>Arabidopsis thaliana</i>	GO:0009735~response to cytokinin, photosynthesis,
AT4G32260	ATPase, F0 complex, subunit B/B', chloroplast(PDE334)	<i>Arabidopsis thaliana</i>	GO:0009735~response to cytokinin, GO:0015986~ATP synthesis coupled proton transport, GO:0015992~proton transport, GO:0042742~defense response to bacterium,



AT5G46110	Glucose-6-phosphate/phosphate translocator-like protein(APE2)	<i>Arabidopsis thaliana</i>	GO:0006281~DNA repair, GO:0008643~carbohydrate transport, GO:0009643~photosynthetic acclimation, GO:0015713~phosphoglycerate transport, GO:0015717~triose phosphate transport, GO:0035436~triose phosphate transmembrane transport,
AT5G66190	ferredoxin-NADP[+]-oxidoreductase 1(FNR1/LFNR1)	<i>Arabidopsis thaliana</i>	GO:0009767~photosynthetic electron transport chain, GO:0042742~defense response to bacterium, GO:0055114~oxidation-reduction process,
AT2G46820	photosystem I P subunit (PSI-P)	<i>Arabidopsis thaliana</i>	GO:0009737~response to abscisic acid, GO:0009773~photosynthetic electron transport in photosystem I,
AT4G12800	photosystem I subunit I(PSAL)	<i>Arabidopsis thaliana</i>	GO:0015979~photosynthesis, GO:0019684~photosynthesis, light reaction,
AT5G06290	2-cysteine peroxiredoxin B (2-Cys Prx B)	<i>Arabidopsis thaliana</i>	GO:0009409~response to cold, GO:0042742~defense response to a bacterium, GO:0045454~cell redox homeostasis, GO:0055114~oxidation-reduction process, GO:0004601~peroxidase activity,GO:0016209~antioxidant activity,GO:0051920~peroxiredoxin activity,GO:0004601~peroxidase activity,GO:0016209~antioxidant activity,GO:0051920~peroxiredoxin activity,
AT3G11630	Thioredoxin superfamily protein	<i>Arabidopsis thaliana</i>	GO:0009409~response to cold,GO:0042742~defense response to bacterium,GO:0045454~cell redoxhomeostasis,GO:0055114~oxidation-reduction process,GO:0004601~peroxidase activity,GO:0005515~protein binding,GO:0016209~antioxidant

			activity,GO:0051920~peroxiredoxin activity,
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#### 4. Discussion

Carbonic anhydrase is a metabolic enzyme found in different concentrations in different plant species. Under high temperature and limited carbon dioxide conditions, the rate of carbon fixation decreases, and as a result, photosynthesis is immediately decreased. In C4 plants, Carbonic anhydrase plays a vital role in fixing CO<sub>2</sub> and converting it into C4 acid (Bhatt et al., 2017).

However, the involvement of CAs in stress tolerance processes has been explored in plants. The participation of more than one CA in one biochemical pathway may explain the lack of understanding of the roles of specific CAs in higher plants with C3 photosynthesis. The lack of CAs in mutants has a more pronounced effect under varied unfavourable situations that are pleasant for plants. Under ideal light intensity, temperature, mineral nutrition, and so forth, CO<sub>2</sub> supply and fixation are not confined to general plant metabolism. Furthermore, the lack of CAs in mutants has a more pronounced effect under various unfavourable situations. The role of CAs in plants under stress is the most critical.

Computational analysis has proved that neighbours of the genes have a significant role in its regulation, and the results of such studies have been validated through experiments as well. Investigating the neighbour proteins of cancer-related genes has uncovered a lot of potential drug targets for cancer as these neighbour proteins significantly regulate the target cancer-related proteins (Módos et al., 2017). This approach has been used in many areas to decrease the cost of wet-lab experiments and narrow down the search for potential influencer genes for the target gene (Módos et al., 2017). In recent times, when a lot of research is underway on photosynthesis under high temperature and low CO<sub>2</sub> conditions to uncover the possibility of transforming plants from C3 to C4 photosynthesis, we have conducted a study to find the role of neighbours of CA1 in carbon fixation during C4 photosynthesis.

We have extracted a BCA1 mediated PPI network of 101 nodes from the STRING database (Mering et al., 2003) and subjected them to Cyto-HUBBA (Chin et al., 2014) within a Cytoscape environment (Shannon et al., 2003) for identification of essential proteins. Cyto-HUBBA is a publicly available java scripted, powerful molecular network visualization and analysis tool that provides additional features for analysing PPI networks (Chin et al., 2014). The tool is used to identify the proteins that have significant interactions with other proteins and plays a crucial role in the up or down-regulation of the proteins in the network. Cyto-Hubba ranks the hub genes based on Maximal Clique Centrality (MCC),

which has the highest efficiency among all the known methods of identifying hub proteins (Chin et al., 2014). The top picks were further prioritized using the Arabidopsis thaliana-specific hub proteins identification tool AraNet v2. AraNet is a computational tool that uses a probabilistic approach to integrate omics data and prioritizes the proteins in the network that have more and probably significant interactions among other proteins in the network (Zhai et al., 2016). The top 34 common proteins from both tools were subjected to GeneMania to identify predicted interactions (Franz et al., 2018). The GeneMania is a freely accessible web-based tool used to construct the PPI network, Physical interactions, shared Domains, Genetic interaction, and Co-localization of Proteins. It is also used to predict the function and genetic interactions of proteins. GeneMania can predict interactions not available in the literature but can be significant in the up and down-regulation of other genes in the network (Franz et al., 2018). The predicted interactions were screened for interactions present among CA1 and proteins involved in the same photosynthetic biological pathway. Six proteins (Photosystem I P subunit (CURT1B: AT2G46820), Photosystem I subunit L (PSAL: AT4G12800), Ferredoxin-NADP[+]-oxidoreductase 1 (FNR1: AT5G66190), ATPase (F0 complex subunit B/B' (PDE334: AT4G32260), Glucose-6-phosphate/phosphate translocator-like protein (TPT: AT5G46110) and Photosystem I subunit H2 (PSAH2; AT1G52230) are directly interacting with CA while one additional interactor protein i.e. 2-cys peroxiredoxin A, (2CPA; AT3G11630/BAS1) is also strongly interacting with CA1. Another protein, 2-cysteine peroxiredoxin B (2CPB: AT5G06290), shows solid indirect interaction with CA. GO enrichment analysis of all the eight proteins was performed using the DAVID tool (Huang et al., 2007). Six of the eight hub proteins, i.e., CURT1B, PSAL, FNR1, PDE334, TPT, and PSAH2, are reported as involved in photosynthesis.

Curvature Thylakoid 1B (CURT1B; AT2G46820) encodes the P-subunit of Photosystem I and is 14kdthylakoid membrane phosphoprotein (TMP14) in Arabidopsis thaliana. CURT1B is the constituent of chloroplast and is involved in photosynthetic electron transport in photosystem I. It is also involved in response to abscisic acid (Trotta et al., 2019).

Photosystem I subunit L (PSAL; AT4G12800) encodes subunit L of the Photosystem I reaction centre and is in the chloroplast. It is involved in the GO biological process of Photosynthesis and performs the molecular function of protein binding. It is expressed during the growth and development of seedlings, leaves, and flowers (Seok et al., 2014).

Ferredoxin-NADP (+)-Oxidoreductase 1 (FNR1; AT5G66190), also known as leaf-type chloroplast-targeted FNR 1 (LNFR1), encodes a leaf-type ferredoxin: NADP(H) oxidoreductase which is involved in the photosynthetic electron transport chain and

performs the function of Poly(U) RNA binding during NADPH dehydrogenase activity. It is found in both the thylakoid and stroma of chloroplast but is more abundant in thylakoid. It shows affinity towards ferredoxin and, in combination with FNR2, makes homodimer a prerequisite to attaching FNR2 to the thylakoid membrane (Lintala et al., 2012).

Pigment Defective 334 (PDE334; AT4G32260) is a chloroplastic ATPase enzyme that responds to cytokinin during ATP synthesis coupled with proton transport. It performs the molecular function of Proton transport during ATPase activity (Friso et al., 2004).

Acclimation of Photosynthesis to Environment 2 (APE2; AT5G46110) is also known as Triose Phosphate Translocator (TPT) and encodes a chloroplast triose phosphate. The latter is involved in photosynthetic acclimation transmembrane transport, and the transportation of triose phosphate derived from the Calvin cycle in the stroma to the cytosol to use in sucrose synthesis and other biosynthetic processes. It is found in the chloroplast, Golgi bodies, plasma membrane, and plastid (Weise et al., 2019).

Photosystem I subunit H2 (PSAH2; AT1G52230) is located in the chloroplast and is involved in response to cytokinin during photosynthesis. The phosphorylation of this protein is dependent on calcium; however, the molecular function is still unknown (Varotto et al., 2002).

2-cys peroxiredoxin A (2CPA; AT3G11630/BAS1) contains two catalytic residues that function as a redox cascade with TrxL2 via the ferredoxin-thioredoxin reductase (FTR)/thioredoxin (Trx) pathway to mediate the light-responsive reductive control of target proteins. It continuously transfers reducing power from TrxL2 to H<sub>2</sub>O<sub>2</sub> (Yoshida et al., 2018).

2-cysteine peroxiredoxin B (2-CPB; AT5G06290) is located in the chloroplast and has two catalytic cysteine residues involved in cell redox homeostasis and response to oxidative stress. The carbon dioxide fixed by Carbonic anhydrase during C<sub>4</sub> photosynthesis is also carried out in acute stressed conditions (Broin et al., 2002).

However, our computational analysis has proved one among the hub proteins, i.e., 2CPB; AT5G06290, and an additional interactor protein, i.e., 2CPA; AT3G11630/BAS1, is not previously reported to be involved in any of the biological processes related to photosynthesis. These two genes have specific Carbonic anhydrase interactions and can play a significant role in carbon fixation. It may regulate beta Carbonic anhydrase, i.e., CA1, during C<sub>4</sub> photosynthesis. It may also play a crucial role in transforming plants from C<sub>3</sub> into C<sub>4</sub> photosynthesis.

## 5. Conclusions

The main objective of this study was to decipher the significance of the predicted interactions among the Hub proteins of the Carbonic anhydrase mediated protein-protein

interaction network. Our computational analysis has confirmed six genes, i.e., CURT1B, PSAL, FNR1, PDE334, TPT, and 2CPB, the functional and biological relevance of which suggests that these genes can potentially co-operate with Carbonic anhydrase in its role. DAVID GO biological processes analysis revealed that five of the six genes are already directly or indirectly involved in photosynthesis. However, we have identified a novel gene, i.e., 2-cysteine peroxiredoxin B (2-CPB; AT5G06290), that interacts significantly with Carbonic anhydrase and can regulate CA in *Arabidopsis thaliana* during C4 photosynthesis. The functional analysis of this gene also showed that it has a role under oxidative stress. It may be concluded that 2-cysteine peroxiredoxin B (2-CPB; AT5G06290) may have a role in Carbon dioxide fixation by regulating Carbonic anhydrase. Further, its role may be significant in transforming C3 into C4 photosynthesis.

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