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In Silico Interactome Network Analysis and Phylogenetic Relationship of Potato Peroxidases and Catalases

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ABSTRACT

Peroxidases (POXs) and Catalases (CATs) are the main antioxidant enzymes involved in scavenging H_2O_2 in living cells. Different POXs and CATs may be capable of exhibiting interaction with the constituents of the plant cell. Whereas the activity or gene expression of POXs and CATs has been investigated in potato plants, their interactions with other proteins in this crop have not been investigated till now. Determining Protein-Protein Interaction (PPI) networks could be important in providing crucial insights into the regulation of plant defense responses to biotic and abiotic stresses. STRING analysis revealed interaction of cationic, suberization-associated anionic, and Class III peroxidases in potato with several enzymes involved in lignin biosynthesis and phenylpropanoid pathways, which was in accordance with close phylogenetic relationship of the three potato peroxidases investigated in this study. The CAT1 enzyme in potato interacted with several enzymes involved in ROS production. Phylogenetic analysis of the *CAT1* and *CAT2* genes in this plant species referred to their close relationship. Demonstrating how each isoform of these enzymes responds to environmental stimuli and how it interacts with other proteins at transcriptional, translational, and post-translational levels seems to be useful in designing novel and effective plant protection strategies against different stresses.

Keywords: Defense mechanisms, Interactome, Plant protection strategies, **P**rotein-protein interaction.

INTRODUCTION

Resistance mechanisms, including numerous biochemical and cytomolecular changes, occur in plant defense against biotic and abiotic stresses. Defense signaling is complex and comprises mechanisms which distinguish a range of environmental stimuli. Accumulation of Reactive Oxygen Species (ROS; including Hydrogen peroxide: H_2O_2, Superoxide: O_2^-, and Hydroxyl radical: OH^-), known as oxidative burst, is one of the earliest plant defense responses to various biotic or abiotic stresses. Various types of ROS can enhance Hypersensitivity Response (HR) or play an important role as second messengers in resistance mechanisms leading to the upregulation of defense-related genes and interaction with other signaling molecules (Chen, *et al.* 2013). Previously, it had been assumed that various ROS accumulate sequentially from O_2^- as the primary origin. Today, we know that different ROS can be produced independently by different sources, which seems reasonable because ROS accumulation must be under control of antioxidative systems to avoid toxicity (Hückelhoven and Kogel 2003). All aerobic organisms have developed antioxidant systems for controlling ROS accumulation and maintaining redox homeostasis, as well as to 'make use' of these highly reactive molecules in signal transduction, gene expression and cellular responses to biotic or abiotic stimuli

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(which is redox signaling). Uncontrolled ROS levels lead to cell death, which may enhance plant susceptibility to pathogens (Torres, *et al.* 2006). So, ROS accumulation and removal are controlled in plant-pathogen interactions. Enzymatic antioxidants such as Peroxidase (POX) and Catalase (CAT) are responsible for scavenging H_2O_2 in living cells (Barna *et al.* 2012).

The POXs are α- helical heme-containing proteins with various functions. They might be involved in H_2O_2 production (peroxidation) or in oxidizing various molecules mainly of phenolic nature. Peroxidases (EC 1.11.1.7) are oxidoreductases that catalyze the reduction of hydrogen peroxide and the concomitant oxidation of various hydrogen donors, such as phenolic and non-phenolic substrates (Carpin, *et al.* 2001). Most of the functions recognized for plant peroxidases occur in cell walls. These functions can be divided into two major types. The first is the oxidative cross-linking of several aromatic molecules and structural non-enzymatic proteins such as extensins via using H_2O_2 as an electron acceptor. This reaction leads to lignification (Ros Barceló *et al*. 1998) or production of suberin by the function of class III secretable plant peroxidases (Bernards 1999; Arrieta-Baez and Stark 2006) and also to cell wall reinforcement via formation of covalent bonds between polysaccharides such as pectin and hemicellulose (Fry 1986; Passardi, *et al.* 2005).

On the other hand, some POXs such as class III secretable POXs are involved in peroxidation by producing ROS including H_2O_2, O_2^-, and OH^- (Bestwick, *et al*. 1999; Raggi *et al.* 2015). H_2O_2 and O_2^- are used in a Fenton-type reaction to produce OH^-, which leads to non-enzymatic cleavage of cell wall polysaccharides (Passardi *et al.* 2005; Raggi *et al.* 2015). Therefore, the POXs are capable of playing opposite functions in cell expansion, by causing both wall thickening and loosening via negatively or positively regulating ROS accumulation, depending on growth conditions (Passardi,*et al.* 2004).

Many different isozymes of POXs are present simultaneously in cell wall and other apoplastic spaces (Raggi, *et al.* 2015; Ros Barcelö, *et al.* 1989), cytoplasm (Ros Barcelö, *et al.* 1989), and vacuoles (Calderon, *et al*. 1992). These enzymes have been grouped in three different categories, including soluble, ionically bound, and covalently bound POXs, depending on the treatment necessary for their release from the plant cell wall (McDougall *et al.* 1995). This suggests that different POXs may be capable of exhibiting various interactions with the constituents of the plant cell or its extracellular matrix. Despite the probable importance of these interactions for the control of POX function, the current information in this case is scarce.

The CAT (EC 1.11.1.6) is a tetrameric heme protein with four polypeptide chains, which has α-helix and β-sheet domains. It is found in all aerobic living organisms and functions in converting H_2O_2 to H_2O and O_2 (Chelikani *et al.*, 2004). This enzyme is also found in some anaerobic microorganisms such as *Methanosarcina barkeri* (Brioukhanov *et al.* 2006). It is a main enzymatic antioxidant, which protects the cells from harmful damages of oxidative busrt. This enzyme has one of the highest turnover numbers among all enzymes, as one molecule of CAT can scavenge millions of H_2O_2 molecules in each second (Goodsell, 2016). Catalase is located in the peroxisomes of eukaryotic cells (Alberts *et al.*, 2002.). Peroxisomes in plant cells are involved in photorespiration (the use of O_2 and production of CO_2) and symbiotic nitrogen fixation (the breaking apart of N_2 to reactive nitrogen atoms). Plant catalases can be classified into three classes based on the expression profiles of the *CAT* genes (Willekens, *et al*. 1994; Willekens *et al*. 1995). Class I of catalases includes isoforms which are highly expressed in leaves and involved in the removal of H_2O_2 produced during photorespiration. The class II of catalases includes the isoforms which are generally found in vascular tissues and its physiological role is unclear, so far (Dat *et al*. 2000; Feierabend 2005). The class III of catalases includes isoforms which are expressed in seeds and young seedlings and

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believed to be involved in the removal of H_2O_2 during fatty acid degradation in glyoxysomes (Willekens, *et al*. 1994; Willekens *et al*. 1995).

Both CATs and POXs are involved in protecting living cells and tissues against toxic effects of ROS, including oxidative damage of DNA, proteins, and lipids (Halliwell and Gutteridge, 1989; Novo and Parola 2008). Whereas the activity or gene expression of POXs and CATs at different stress conditions have been investigated in potato plants, their interactions with other proteins in this crop plant is not investigated till now. Determining Protein-Protein Interaction (PPI) networks, which is also known as interactome (Braun *et al*. 2013), could be important in providing crucial insights into the regulation of plant developmental, physiological, pathological and defense-related processes against various biotic and abiotic stresses. Additionally, any interaction between plant proteins and pathogenic organism proteins will also increase understanding of the plant proteome and defense networks against pathogens.

In potato (*Solanum tuberosum*), various types of POX, such as cationic peroxidase (poxMM), suberization-associated anionic peroxidase, lignin-forming anionic peroxidase, phospholipid hydroperoxide glutathione peroxidase, ascorbate peroxidase, cell wall peroxidase, thioredoxine peroxidase, class III peroxidases and several isoenzymes of peroxidase are identified, so far (Espelie and Kolattukudy, 1985; Yu, *et al.* 2010). Three isozymes of CAT, including CAT1, CAT2, and CAT3 are reported in potato till know (Santos *et al.* 2006).

Interactome analysis methods could be classified into three groups, including *in vitro*, *in vivo*, and *in silico* approaches. Bioinformatics predictions, also referred to as *in silico* analysis, can suggest interactions that have avoided recognition by other methods or those of proteins that have not been investigated yet.

The objectives of this study were to: (i) Investigate the interactome for each of the main peroxidases (including the cationic peroxidase poxMM, suberization-associated anionic peroxidase POX, and a member of Class III of POXs) in potato, (ii) Determine interaction networks for each of the three isozymes of CAT in this plant species, (iii) Predict interaction of these antioxidant proteins with each other via *in silico* analysis, and (iv) Determine phylogenetic relationships of the POX and CAT isozymes in potato for getting more insight about unknown functions and possible interactions of these important redox-related enzymes.

MATERIALS AND METHODS

Bioinformatic Analysis of Protein-Protein Interaction Networks.

Interaction of CAT1, CAT2, CAT3, poxMM, suberization-associated peroxidase (POX) and a peroxidase belonging to Class III of POXs (CIIIPOX) with their predicted functional partners in potato was investigated based on bioinformatic analyses by the Search Tool for the Retrieval of Interacting Genes (STRING) software version 10 (Szklarczyk *et al.*, 2015). Furthermore, the interactome of the above mentioned CATs and POXs with each other was analyzed using the same software.

Sequence Analysis and Construction of Phylogenetic Tree.

Sequences of the *poxMM*, *CAT1* and *CAT2* genes of potato were obtained from NCBI and compared with other *peroxidase* and *catalase* genes in *Arabidopsis thaliana*, *Solanum lycopersicum* (tomato), and *Oryza sativa* (rice). Sequence alignments and editions were performed using the BioEdit Sequence Alignment Editor (Tom Hall, Ibis therapeutics, Carlsbad, CA, USA). Then, the phylogenetic trees were constructed using Treecon software (Van de Peer, and

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Wachter, 1994). Bootstrap re-samplings with 1,000 trials were used for estimating the confidence degree in the clustering order of phylogenetic tree obtained from the sequences.

RESULTS

Bioinformatic Analysis of Protein-Protein Interactions

For detailed explanation of a protein's function in basal resistance, knowledge about its interaction associates is a major prerequisite. STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) is one of the best sites to hold experimental, predicted and transferred protein-protein associations, together with interactions obtained through text mining. Using the STRING v10 database (http://STRING-db.org/), we investigated the known and predicted interactions among each of the poxMM, POX, CIIIPOX, CAT1, CAT2, and CAT3 with other proteins in potato. In addition, interaction of these proteins with each other was investigated. The protein networks consist of information concerning both nodes (proteins) and edges (interactions). Each node in the network shows a preview to 3D protein structure, and can be clicked to reveal a pop-up window with more information about the protein including its annotation, structure, and homology (Szklarczyk *et al*. 2015). Protein nodes that are enlarged indicate the availability of 3D protein structure information. Each edge in the obtained networks represents a known or predicted interaction, and leads to a pop-up window with information about the basic evidence and the interaction confidence scores. Colored lines between the proteins indicate different types of interaction. According to the STRING analysis, cationic peroxidase (poxMM) in potato had interaction with glycosyltransferase, aminotransferases, cinnamyl alcohol dehydrogenase, alcohol dehydrogenase and the isozymes of phenylalanine ammonia-lyase (Figure 1-a). However, no information about co-expression of the *poxMM* with other genes of this network was found in database.

Suberization-associated anionic Peroxidase (POX) interaction analysis based on databases (Figure 1-b) revealed the association of this protein with the same proteins that were found in the interactome of poxMM. In addition, the same proteins were found in the interactome of Class III Peroxidase (CIIIPOX) in potato (Figure 1-c). Interaction analyses data obtained for all three types of potato peroxidases tested in this study were based on databases and no evidence of neighborhood, gene fusion, and coexpression was observed in the interactome of these proteins (Figure 1).

Bioinformatic analysis revealed that the CAT1 in potato had interaction with Peroxisomal Targeting Signal (PTS) 1 receptor, Glycolate Oxidases (GOX), (s)-2- hydroxy-acid oxidases, Superoxide Dismutase (SOD), and trehalose-6-phosphate synthase (Figure 2). The STRING analyses revealed co-expression of the *CAT1* with putative homologs of other genes in the obtained network, except for the *PTS* (Figure 2).

Proteins obtained in the interaction network of CAT1 were also observed in the STRING results for CAT2, except SOD. Co-expression of *CAT2* with all genes in its network was predicted as observed for their putative homologs in other species (Figure 3).

Interaction network of CAT3 in potato consisted of the same proteins, which were observed in the interactome of CAT2 (Figure 4). In the interactome of each catalase investigated, the evidence of coexpression of the gene encoding the main protein of the network (CAT1, CAT2, or CAT3) with the genes associated with other proteins in each network, except the gene of peroxisomal targeting signal 1 receptor, was observed.

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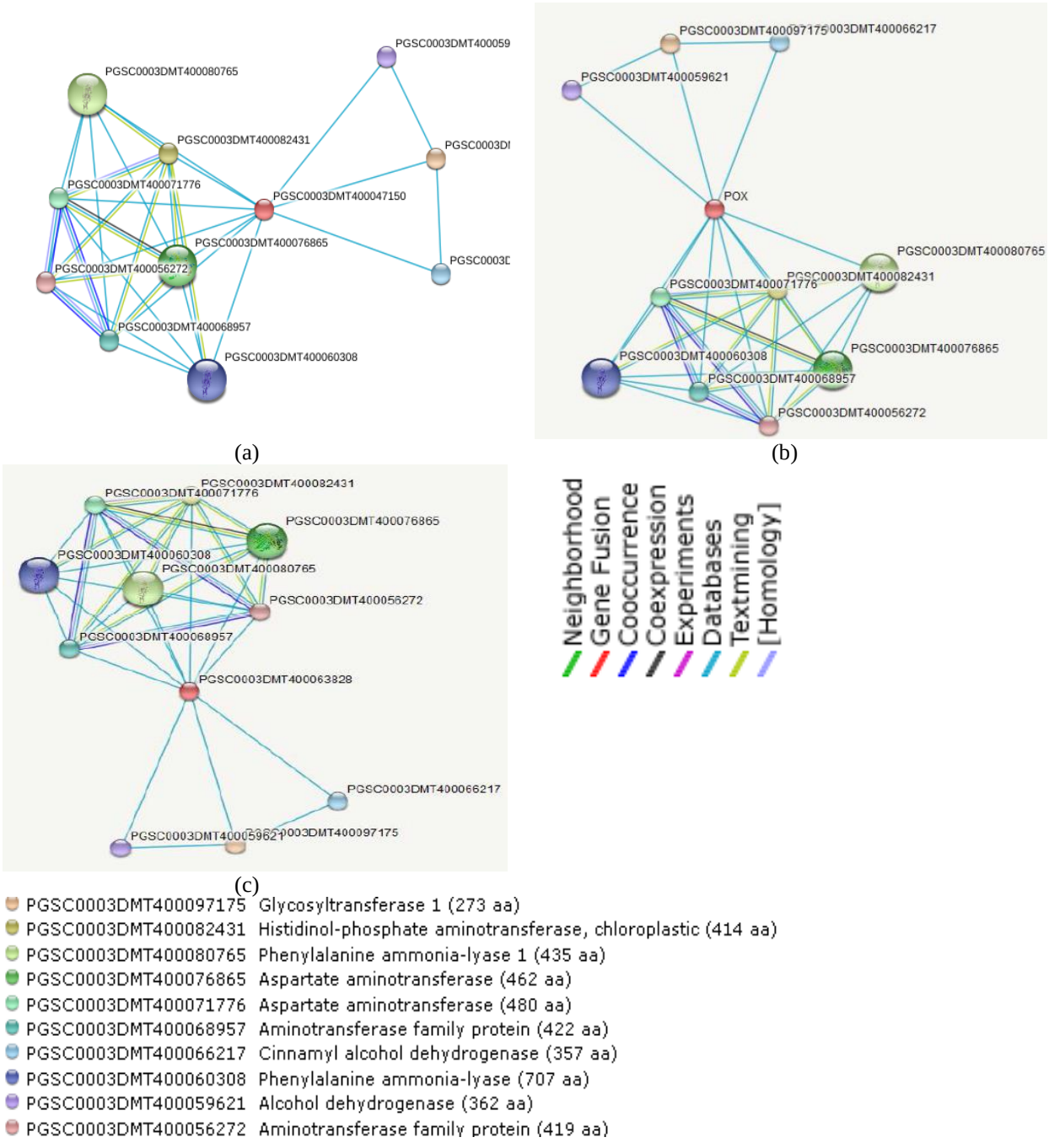

Figure 1. Interaction of cationic peroxidase 1 (a) (poxMM, Identifier: PGSC0003DMT400047150), suberization-associated anionic Peroxidase (b) (POX, PGSC0003DMT400057522), and Class III Peroxidase (c) (CIIIPOX, PGSC0003DMT400063828) with their functional partners in *Solanum tuberosum* based on bioinformatic analysis using STRING software (version 10).

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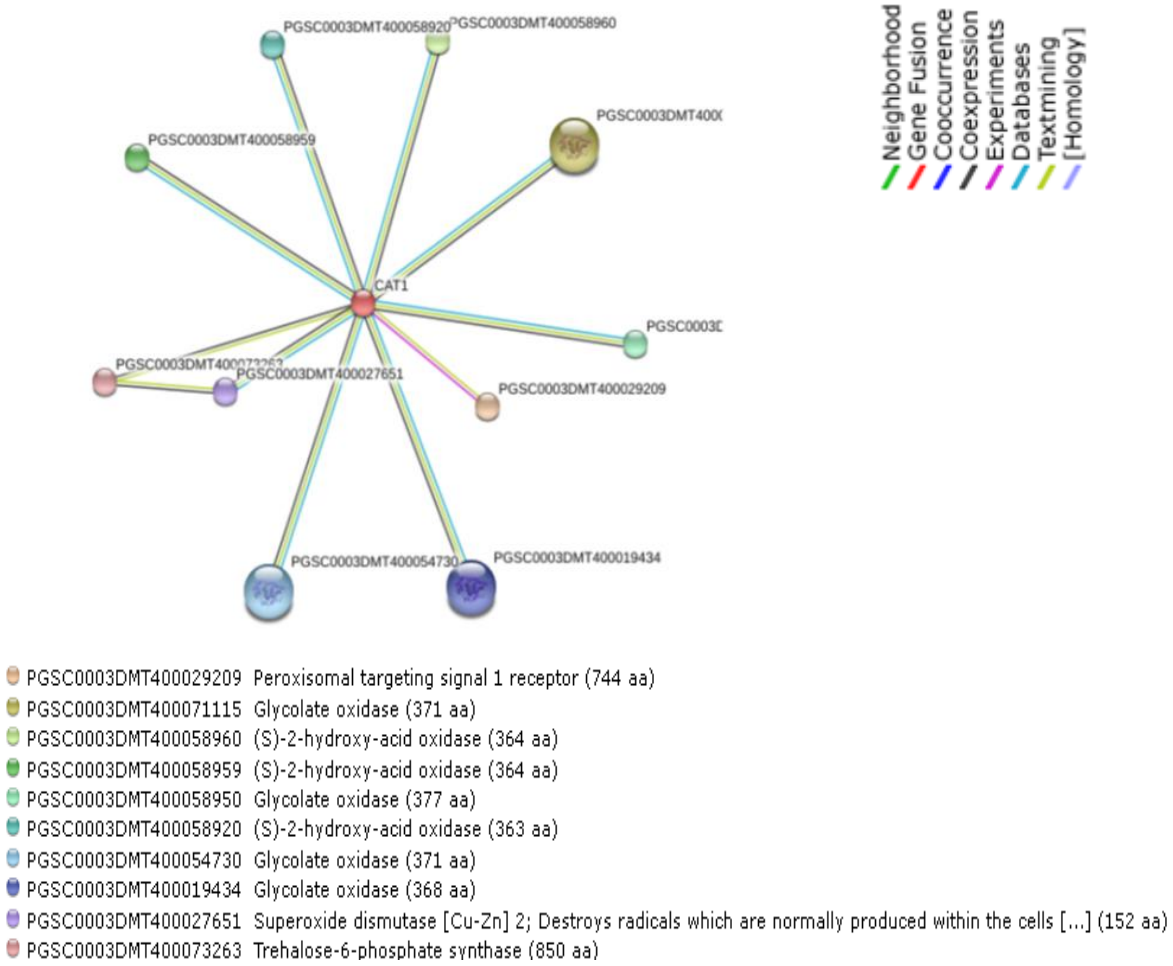

Figure 2. Interaction of Catalase 1 (CAT1, Identifier: PGSC0003DMT400075611) protein with its functional partners in *Solanum tuberosum* based on bioinformatic analysis using STRING software (version 10).

No interactions were observed among the CAT1, CAT2, CAT3, poxMM, POX, and CIIIPOX in *S. tuberosum* (Figure 5-a). Whereas, association analysis of Peroxidases (POXs) in this plant species revealed the presence of interactions among 15 out of 132 POXs (Figure 5-b). As is shown in Figure 5-b, most of the interactions in this network were based on text mining. Association of glutathione peroxidases (169 and 170 aa), thioredoxin peroxidases (162, 267, and 272 aa), and an ascorbate peroxidase (287 aa) was also observed based on the data obtained by various experiments, which are shown as pink lines. In addition, coexpression might occur in the interaction of the gene encoding thioredoxin peroxidase 1 (169 aa) with the genes associated with each of the ascorbate peroxidase (287 aa), phospholipid hydroperoxide glutathione peroxidase (169 aa), glutathione peroxidase (170 aa), and thioredoxin peroxidases (267 and 272 aa). Coexpression of the thylakoid-bound ascorbate peroxidase with thioredoxin peroxidases was also observed in Figure 5-c.

Comparative Molecular Phylogeny of the *CAT* and *POX* Genes in Potato and other Plants.

To better understand POX and CAT sequence variance and similarities among potato genes and other plants, a phylogenetic tree was constructed using multiple

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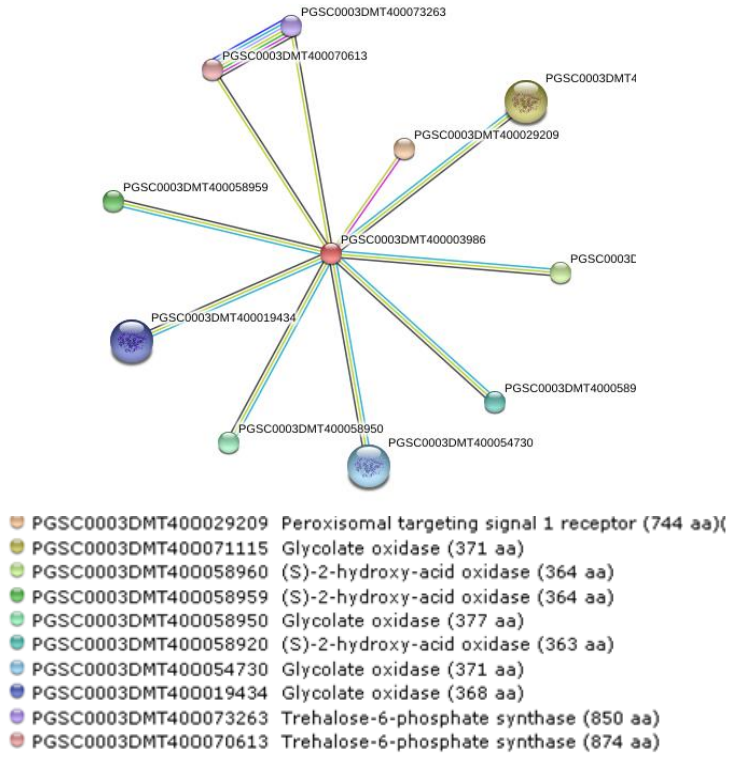

Figure 2. Interaction of Catalase 1 (CAT1, Identifier: PGSC0003DMT400075611) protein with its functional partners in *Solanum tuberosum* based on bioinformatic analysis using STRING software (version 10).

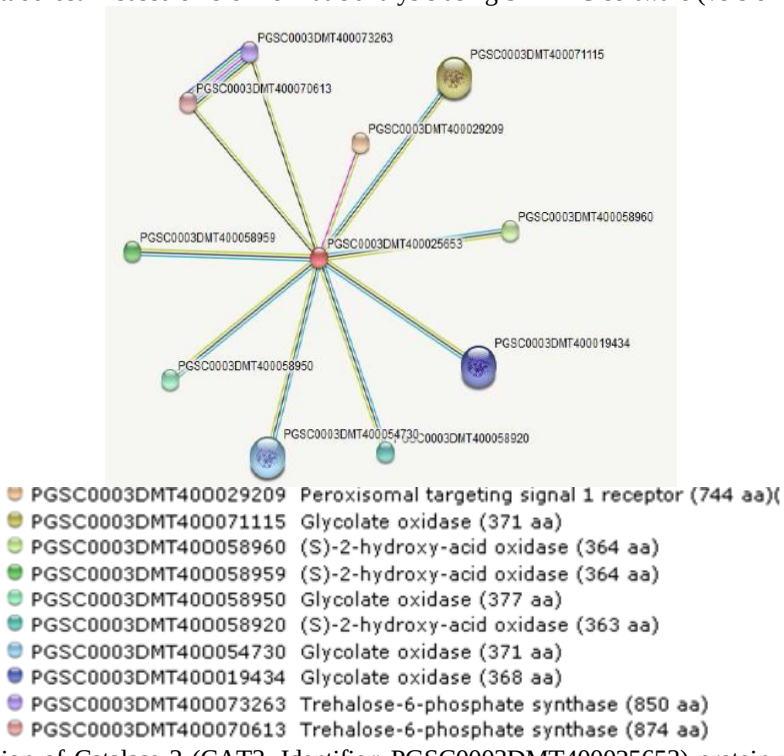

Figure 4. Interaction of Catalase 3 (CAT3, Identifier: PGSC0003DMT400025653) protein with its functional partners in *Solanum tuberosum* based on bioinformatic analysis using STRING software (version 10).

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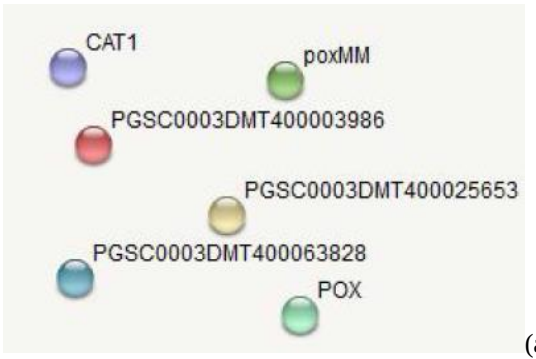
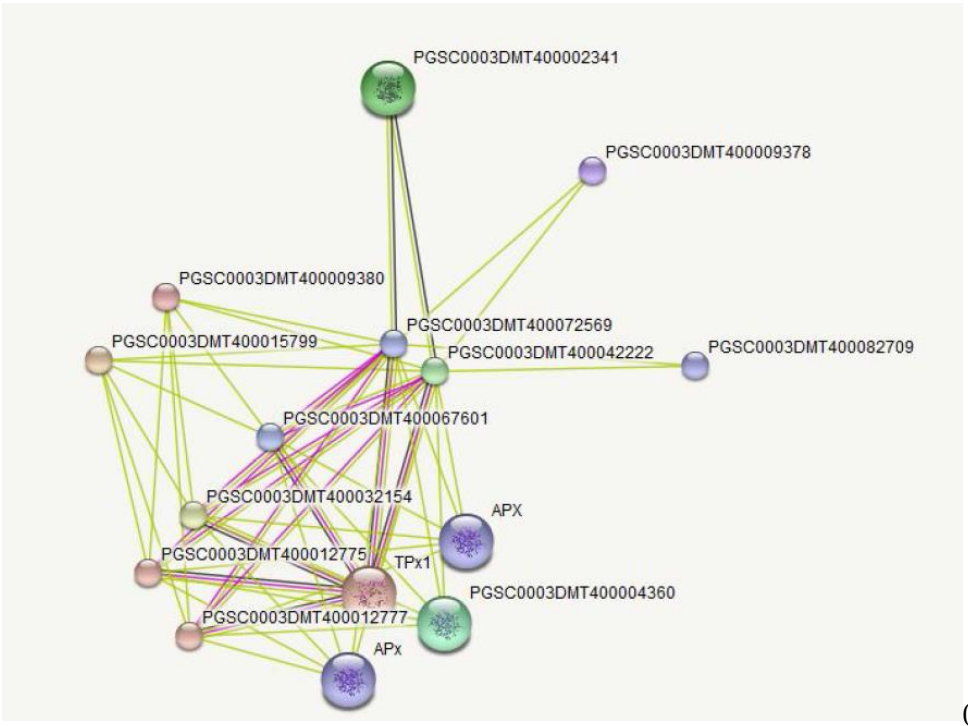


(a)



(b)

Figure 5. Interactome of catalases and peroxidases in potato. (**A)** Interactome of Catalase 1 (CAT1), Catalase 2 (CAT2), Catalase 3 (CAT3), cationic peroxidase 1 (poxMM), suberization-associated Peroxidase (POX), and Class III Peroxidase (CIIIPOX) based on bioinformatic analysis using STRING software (version 10). **(B)** Interactome of Peroxidases (POXs) in potato (15 out of 132 POXs showed interaction). Edges represent protein-protein associations are meant to be specific and meaningful, i.e. proteins jointly contribute to a shared function; this does not necessarily mean they are physically binding each other.

sequence alignment. Phylogenetic tree revealed that the cationic peroxidase of Potato (poxMM) and suberization-Associated Peroxidase (POPA) were clustered in a group together with the TMP1 and TAP1 genes, which are anionic peroxidases in tomato. Other Tomato Peroxidases (TPX1, TPX2, and pox3) were grouped together in the next cluster and the rice Cationic Peroxidase (POC1), which was used as an out group, was clearly separated from other peroxidase genes analyzed (Figure 6a).

Interestingly, the *CAT1* and *CAT2* genes in potato clustered together with high bootstrap value of 92 percent and showed high similarity with isoforms of CAT1 in tomato. The *catalase* genes of *Arabidopsis* had high similarity with the tomato CAT2 and the CAT of a japonica cultivar of rice (Figure 6-b).

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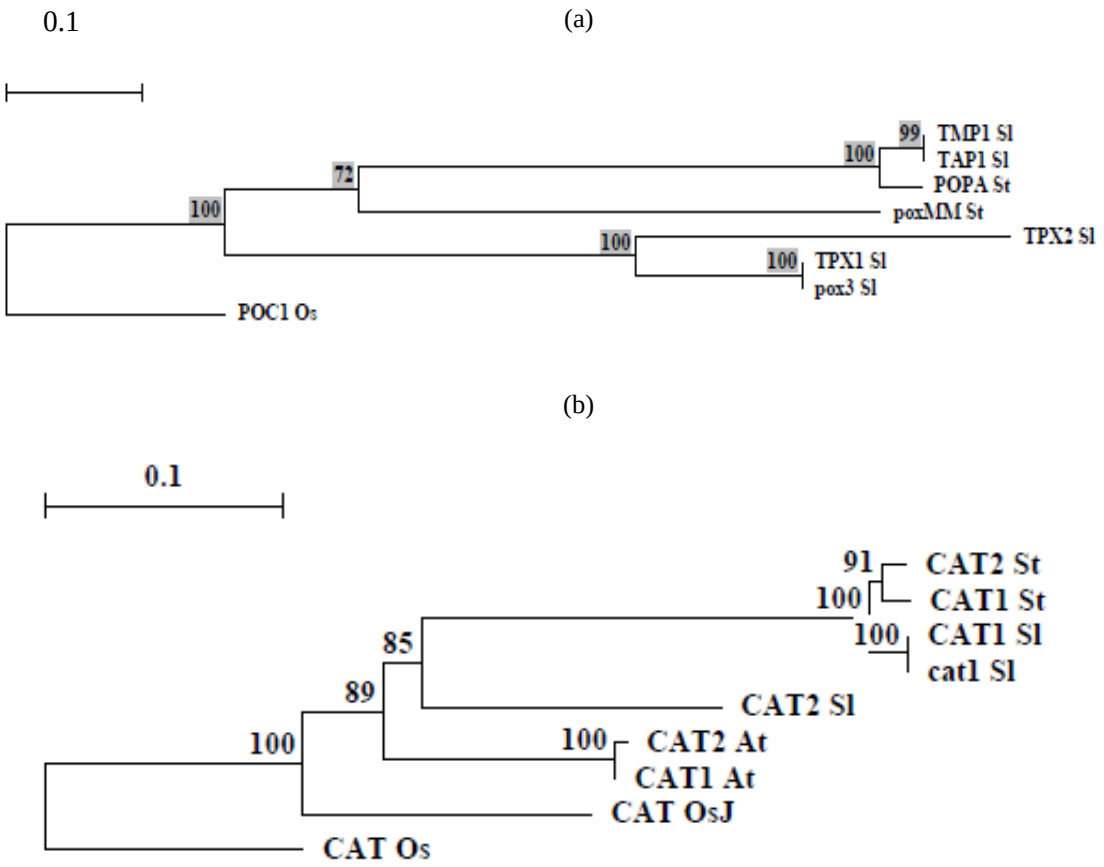

Figure 6. Rooted neighbor-joining phylogenetic trees of plant peroxidases (**A**) and catalases (**B**) constructed in Treecon software. The gene names are mentioned next to the branches followed by abbreviation of the scientific name of corresponding plant species. Sl, *Solanum lycopersicum*; St, *S. tuberosum*; Os, *Oryza sativa*; OsJ, *O. sativa* Japonica cultivar; At, *Arabidopsis thaliana*. The complete sequence of other plant peroxidases and catalases were not in the NCBI database. GenBank accession numbers of the genes are depicted after the gene names here: *TMP1* Sl, NM_001309316; *TAP1* Sl, X15853, POPA St (Suberization–associated peroxidase), XM_006347106.2; *poxMM* St, DQ925471.1; *TPX2* Sl, L13653.1; *TPX1* Sl, L13654.1; *pox3* Sl, NM_001302921; *POC1* Os, AF247700; CAT2 St, AY500290; CAT1 St, U27082; cat1 Sl, M93719.1; CAT1 Sl, NM_001247898.1; CAT2 Sl, NM_001247257; CAT1 At, AY054663; CAT2 At, X64271.1; CAT OsJ, D2648.1; CAT Os, AB020502.

DISCUSSION

Proteins control all biological systems in a cell, and as many proteins perform their roles independently, the vast majority of proteins interact with others for proper biological activities. Investigating protein-protein interactions by bioinformatics or via various *in vitro* and *in vivo* methods (reviewed by Rao *et al*. 2014) is critical to understand protein functions in plant defense responses.

A very good software for data integration and analysis of protein-protein interaction networks (in terms of methods, resources, and graphical display) is the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) (Szklarczyk, *et al.*, 2011). This database combines and analyzes several data points to predict functional linkages and

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physical interactions between proteins. It takes extensive advantage of genome context methods (gene neighboring, gene split/fusion, and phylogenetic profile), clusters of orthologous genes, coexpression data, experimental and predicted knowledge obtained from biological databases, and the literature to assign a probabilistic confidence score to each functional association (Braun *et al*. 2013). Primarily, the STRING database was focused on prokaryotes, but now it manages more than 1,000 sequences not only from prokaryotes but also for eukaryotes such as fungi, animals, and plant species including potato.

According to the STRING analysis, cationic peroxidase (poxMM) in potato had interaction with several genes/proteins involved in lignin biosynthesis and phenylpropanoid pathways, such as cinnamyl alcohol dehydrogenase, alcohol dehydrogenase, and the isozymes of phenylalanine ammonia-lyase. In addition, the poxMM had association with glycosyltransferase, which strongly controls phenylpropanoid pathway (Aksamit-Stachurska *et al.* 2008.), and also with aminotransferases, which are involved in phenolics production (Ma, *et al.* 2013; Tohge, *et al.* 2013). However, any information about co-expression of the *poxMM* with other genes of this network was not found in the database, which could be the subject of future researches in this plant species.

The same proteins that were found in the interaction network of poxMM were also observed in the interactomes of suberization-associated anionic Peroxidase (POX) and Class III Peroxidase (CIIIPOX) in potato. This data indicated similar functional properties and close relationship of the three potato peroxidases investigated in this study. In the phylogenetic tree, the *poxMM* showed high similarity to the *TAP1* of tomato that is involved in suberization. So, the *poxMM* might be involved in suberization or other phenolics production in potato cells that should be investigated in future researches.

Plants contain multiple forms of catalase, which may reveal various functions of this antioxidative enzyme (Feierabend, 2005). Higher plants generally have three main isoforms of CAT including CAT1, CAT2, and CAT3 (Feierabend, 2005; Heinze and Gerhardt 2002). The CAT1 in potato had interaction with superoxide dismutase (SOD) as a potent scavenger of O_2^-. Both CAT1 and CAT2 had association with various isozymes of Glycolate Oxidase (GOX) as alternative sources for H_2O_2 production in peroxisomes, which led to callose deposition (Rojas *et al.*, 2012.) that is a main defense response against several phytopathogens (Hukkanen, *et al.* 2007; Noorbakhsh and Taheri, 2016). Interaction of both the CAT1 and CAT2 with peroxisomal targeting signal 1 receptor, (s)-2- hydroxy-acid oxidase, and trehalose-6-phosphate synthase was also observed in the STRING analysis. All peroxisomal proteins, such as GOX, are synthesized in the cytoplasm and then must be directed to the peroxisome. The first step in this process is binding the protein to a receptor, which directs the complex to the peroxisome. The receptors bind to a region of the peroxisomal protein that is called Peroxisomal Targeting Signal (PTS). The (s)-2- hydroxy-acid oxidase, found in the interaction network of both CAT1 and CAT2, is involved in phenolics production pathway (Khadem and Marles, 2010). Trehalose-6-Phosphate Synthase (TPS), which was associated with both CAT isozymes in potato, is an important enzyme in the trehalose biosynthetic pathway. Trehalose contents, which play a key function in metabolic regulation and plant resistance to biotic and abiotic stresses, are modulated by the TPS (Xie *et al.* 2015). So, detailed investigations on the role of TPS in potato defense against various destructive fungal and Oomycete pathogens such as *Alternaria* spp., *Rhizoctonia solani*, *Phytophthora infestans* and its association with *in planta* levels of ROS, enzymatic and non-enzymatic antioxidants could be an interesting subject of future investigations. The CAT1 of potato

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is classified in class I of catalases, which are involved in removing H_2O_2 from plant cells, whereas potato CAT2 belongs to Class II of catalases, with a role in stress protection and lignin formation (Almeida *et al.* 2005). Presence of the same proteins in the interaction networks obtained for CAT2 and CAT3 in potato suggested that the isozymes of CAT might have similar functions. It is known that CAT3 is a powerful H_2O_2 scavenger during degradation of fatty acids in glyoxysomes (Willekens, *et al*. 1994; Willekens, *et al.* 1995). Therefore, CAT2 in potato might be involved in this process, which needs to be investigated experimentally. Bioinformatic analyses in the present work revealed no interaction among different CATs and POXs in potato. Interaction analysis of POXs in potato revealed that 15 out of 132 of these antioxidant enzymes interacted with each other with a complex network. Most of these interactions were obtained via the STRING software using textmining, and some of them by the experimental and coexpression data. So, proving all of the observed interactions among potato POXs using valid in vitro and in vivo experiments seems to be necessary.

Phylogenetic analyses revealed the relationship among various types of peroxidases in potato compared to those of tomato. The fact that suberization–Associated Peroxidase (POPA) in potato was closely related to the anionic peroxidases in tomato, including TMP1 and TAP1, suggested the possibility of anionic nature for the POPA, which needs further investigations.

Our data obtained via phylogenetic analysis of the potato *CAT1* and *CAT2* genes were in accordance with a previous report by (Frugoli *et al.* 1998) referring to their close phylogenetic relationship, whereas, (Santos *et al.* 2006) reported that potato *CAT1* and *CAT2* genes were not phylogenetically closely related. The CAT1 belongs to the class I of catalases and is associated with photorespiration, but CAT2 belongs to the class II of catalases and is associated with senescence and other unknown physiological and cellular processes (Feierabend, 2005; Santos *et al.* 2006). The genes encoding class III of catalases in potato, including isoforms expressed in seeds and young seedlings involved in H_2O_2 removal, were not found in the gene banks. So, investigating their phylogenetic correlation with other *CAT* genes was not possible in this study and remains as a subject for future researches. Knowledge on the role of POXs and CATs in plant responses to various biotic and abiotic stresses, which is studies in several plant species (Noorbakhsh, and Taheri 2016; Touiserkani and Haddad, 2012; Abbasi, *et al.* 2014), and their interaction with other proteins and phylogenetic relationships is essential for analyzing new hypotheses about the mechanisms of ROS detoxification and using these findings for designing novel plant protection methods against various environmental stimuli.

CONCLUSIONS

This study provides information on phylogenetic structure and protein-protein interaction network to understand the critical function of peroxidases and catalases as the main antioxidants of potato, which are involved in plant responses to various biotic and abiotic stresses. Findings obtained in this work present the opportunity for further *in vitro*, *in vivo*, and *in silico* assays, which can provide a clearer demonstration about cytomolecular and physiological roles of peroxidases and catalases in potato. These proteins could be involved not only in the ROS homeostasis, but also possibly in other yet unknown signal transduction pathways during development of different tissues, and through interaction of the host plant with various phytopathogens having different lifestyles. Identification of protein-protein interaction networks could be essential in providing critical insights into the regulation of plant defense responses against different environmental stresses. Additionally, any

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interaction between plant proteins and pathogenic organism proteins will also increase understanding of the plant proteome and defense networks against phytopathogens. Therefore, demonstrating how each isoform of these antioxidant enzymes responds to various environmental stimuli and how it interacts with other proteins at transcriptional, translational, and post-translational levels seems to be useful in designing novel and effective plant protection strategies against harmful agents. The information obtained in this study about POXs and CATs interactome networks and phylogenetic analysis will help researchers in the field of oxidative burst and of biotic and abiotic stresses to design and test new hypotheses about the mechanisms of ROS scavenging and their critical roles in plant defense mechanisms in a more holistic manner.

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آنالیز شبکه برهمکنش و ارتباط فیلوژنتیکی پراکسیدازها و کاتالازهای سیب زمینی

پ. طاهری

چکیده

پراکسیدازها (POXs) و کاتالازها(CATs) از آنزیمهای آنتی اکسیدان عمده دخیل در انهدام H_2O_2 در سلولهای زنده می باشند. انواع مختلف آنزیمهای POX وCAT ممکن است که دارای برهمکنش با اجزای سازنده سلول گیاهی باشند. با این که فعالیت آنزیم یا بیان ژن پراکسیدازها و کاتالازها در گیاهان سیب زمینی مورد بررسی قرار گرفته، برهمکنش آنها با سایر پروتئینها در این گیاه زراعی تاکنون ارزیابی نشده است. تعیین شبکه های برهمکنش پروتئین – پروتئین می تواند در ایجاد دیدگاه های مهمی درمورد نحوه تنظیم پاسخهای دفاعی گیاه به تنشهای ناشی از عوامل زنده و غیرزنده حائز اهمیت باشد. آنالیز STRING نشان داد که پراکسیدازهای کاتیونی، آنیونی مرتبط با تولید سوبرین و گروه سوم پراکسیدازها در سیب زمینی با آنزیمهای متعددی که در بیوسنتز لیگنین و مسیر فنیل پروپانوئید دخالت دارند، دارای برهمکنش می باشند که این یافته ها دارای تطابق با ارتباط فیلوژنتیکی نزدیک این سه پراکسیداز موردبررسی در این پژوهش می باشند. آنزیم CAT1 در سیب زمینی دارای برهمکنش با چندین آنزیم دخیل در تولید گونه های فعال اکسیژن بود. آنالیز فیلوژنتیکی ژنهای*CAT1* و *CAT2* در این گونه گیاهی نشانگر ارتباط نزدیک آنها بود. به نظر می رسد که تعیین نحوه پاسخ هریک از این آنزیمها به محرکهای محیطی و برهمکنش آنها با سایر پروتئینها در سطوح رونویسی، ترجمه و فرایندهای پس از ترجمه، در طراحی استراتژی های نوین و موثر حفاظت گیاهان علیه تنشهای مختلف مفید می باشد.

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