Scientific Note

Glutathione peroxidase genes in ancestral sweet potatoes: genome characterization and bioinformatics analysis[1]

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ABSTRACT

Plant glutathione peroxidases (GPXs) consist of non-heme thiol peroxidases that are vital in maintaining hydrogen peroxide homeostasis and regulating plant environmental stress responses. A comparative genomic analysis of the *GPX* gene family in *Ipomoea trifida* and *I. triloba* using their respective genomes was performed. Six *GPX* genes were identified in each species, which were unevenly located in 4 of the 15 chromosomes of the closest ancestors of the sweet potato genomes (*I. trifida* and *I. triloba*). The presence of gene duplications and positive selection were highlighted, suggesting the evolutionary significance of the *GPX* genes in these species. Based on the phylogenetic analysis, the *GPX* genes of *I. trifida*, *I. triloba*, *Arabidopsis thaliana* and *Oryza sativa* can be classified into four groups (I, II, III and IV). The *in silico* expression analysis in different tissues and development stages revealed tissue-specific expression patterns, hinting at specialized roles for the *GPX* genes in different plant organs. Nonetheless, the *ItfGPX5* and *ItbGPX5* genes were highly expressed in most the studied tissues.

KEYWORDS: Comparative genomics, *Ipomoea* species, gene expression, phylogenetic classification.

RESUMO

Genes da *Glutationa peroxidase* em batatas-doces ancestrais: caracterização do genoma e análise bioinformática

As glutationas peroxidases vegetais (GPXs) consistem em peroxidases não-heme tiol vitais na manutenção da homeostase do peróxido de hidrogênio e na regulação das respostas ao estresse ambiental das plantas. Foi efetuada uma análise genômica comparativa da família de genes *GPX* em *Ipomoea trifida* e *I. triloba*, utilizando-se seus respectivos genomas. Foram identificados 6 genes *GPX* em cada espécie, que estavam localizados de forma desigual em 4 dos 15 cromossomos dos ancestrais mais próximos dos genomas da batata-doce (*I. trifida* e *I. triloba*). A presença de duplicações gênicas e a seleção positiva foram destacadas, sugerindo o significado evolutivo dos genes *GPX* nessas espécies. Com base na análise filogenética, os genes *GPX* de *I. trifida*, *I. triloba*, *Arabidopsis thaliana* e *Oryza sativa* podem ser classificados em quatro grupos (I, II, III e IV). A análise da expressão *in silico* em diferentes tecidos e estágios de desenvolvimento mostraram padrões de expressão específicos de tecidos, sugerindo papéis especializados para os genes *GPX* em diferentes órgãos vegetais. No entanto, os genes *ItfGPX5* e *ItbGPX5* foram altamente expressos na maioria dos tecidos estudados.

PALAVRAS-CHAVE: Genômica comparativa, espécies de *Ipomoea*, expressão gênica, classificação filogenética.

Plants often experience various types of stress (Santos et al. 2022). These include both biotic and abiotic stresses, being the latter one of the main challenges that crops face (Bhat et al. 2020, Santos et al. 2022). Different types of abiotic stress can trigger the production of a large number of reactive oxygen species, which can damage essential molecules, membranes and cell organelles, potentially leading to plant cell death (Waszczak et al. 2018, Zafar et al. 2020). Enzymes of the antioxidant system include superoxide dismutases (EC 1.15.1.1), catalases (EC 1.11.1.6), glutathione peroxidases (GPXs) (EC 1.11.1.11) and peroxiredoxins (EC 1.11.1.15) (Meitha et al. 2020).

Plant GPXs are the main enzymes of the antioxidant defense system that sustain hydrogen peroxide homeostasis and regulate the response of plants to abiotic stress conditions. These enzymes are widely distributed among living organisms, including plants, animals and microorganisms. They mainly neutralize organic peroxides and other reactive oxygen species, maintaining the cellular redox balance (Passaia & Margis-Pinheiro 2015). In general, GPXs in plants contain cysteine at their

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functional sites, whereas GPXs in mammals contain selenocysteine residues as cysteine substitutes (Bela et al. 2015, Islam et al. 2015).

Numerous studies have recently shown that increasing or regulating the GPX enzyme activity and expression, as well as regulating *GPX* genes, can help plants to cope with various environmental stresses (Zhou et al. 2018, Wang et al. 2021, Wang et al. 2022). GPXs can be located in different subcellular compartments in plants, including the nucleus, mitochondria, chloroplasts, plasma membrane, cytosol and apoplast (Herbette et al. 2007, reviewed by Margis et al. 2008). Members of the GPX family have been identified and characterized in many plants, including *Thellungiella salsuginea* (Gao et al. 2014), *Gossypium hirsutum* (Chen et al. 2017), *Phoenix dactylifera* L. (Jana & Yaish 2020), *Brassica napus* L. (Li et al. 2021), *Cyprinus carpio* (Xue et al. 2022), *Ammopiptanthus nanus* (Wang et al. 2022), *Capsicum annuum* L. (Wang et al. 2023) and *Cicer arietinum* L. (Parveen et al. 2024).

Eight *GPX* genes in *Arabidopsis thaliana* have been described, and these genes are regulated by abiotic factors (Islam et al. 2015, Bela et al. 2018). The same authors concluded that *GPX* genes may play an essential role in plants under stress. Five *CaGPX* genes were identified in *Capsicum annuum* L. using a bioinformatics method, further highlighting the function of these genes in response to abiotic stress (Wang et al. 2023).

Sweet potato [*Ipomoea batatas* (L.) Lam., 2n = 6x = 90] is an important plant belonging to the Convolvulaceae family, and this crop is grown in more than 100 countries worldwide (Liu 2017). Sweet potato is a vital global food rich in antioxidants and fiber (Alam 2021). However, pests, diseases and environmental stresses hinder its production (Wang et al. 2019). An understanding of its adaptation mechanisms is essential for developing stress-tolerant varieties.

Genomes of the hexaploid sweet potato and two diploid species, namely *I. trifida* NCNSP0306 (2n = 2x = 30) and *I. triloba* NCNSP0323 (2n = 2x = 30) (Wu et al. 2018), were recently made available, facilitating the identification and characterization of specific genes. The *GPX* gene family has not been documented in the sweet potato ancestors *I. triloba* and *I. trifida*. Therefore, the present study was performed to obtain more information about the *GPX* genes in *I. triloba* and *I. trifida*. *In silico* analyses were performed through physicochemical characterization, genetic structure analysis, conserved motif identification, phylogenetic relationship assessment and functional transcriptional profiling using the data obtained from each genome.

The study was conducted at the Universidade do Oeste Paulista, in Presidente Prudente, São Paulo state, Brazil, in 2023.

The protein sequence of *A. thaliana* (AT2G25080.1) was used as a query to identify putative sequences. All predicted amino acid, genomic and coding DNA sequences were recovered from GPX using the sweet potato database. Then, for confirmation, all sequences were selected and submitted to the database of the National Center for Biotechnology Information (Altschul et al. 1997), using the Basic Local Alignment Search Tool for proteins (BLASTP) tool. The identified *GPX* genes were named with the prefix of each species (Itb for *I. triloba* and Itf for *I. trifida*), followed by their chromosomal order. A physicochemical characterization using the Protein Parameters (PROTPARAM) tool of the Expert Protein Analysis System (ExPASy) was also performed, determining the molecular weight (kDa), isoelectric points and gene lengths of the ItlbGPX and ItfGPX proteins. The grand average of hydropathy (GRAVY) value of the protein sequences was determined using the GRAVY calculator. Further, the subcellular locations of the identified proteins were predicted using the Plant-mPLoc server (Chou & Shen 2010). All identified *GPX* genes were named according to their locations and orders on the chromosomes.

All gene structures and positions, including introns and exons, were analyzed using the Gene Structure Display Server (Hu et al. 2015). This tool uses the genomic and coding sequence of each gene to generate the corresponding figure. Sequence motifs were identified and analyzed using the Multiple Em for Motif Elicitation (MEME) web server (Bailey et al. 2009). The number of motifs was set to 10, while all other parameters were set to default values.

The physical locations of the *GPX* genes were obtained from the Sweet Potato Database, and the chromosome location map was constructed using the Mapchart software (Voorrips 2002). The synonymous (*Ks*) and non-synonymous (*Ka*) substitution rates of the paralogous genes were investigated using the Ka_Ks Calculator 2.0 (Zhang et al. 2006). Amino acid sequences of all GPX proteins were aligned

using the ClustalW software (Thompson et al. 1994) with the default parameters. A phylogenetic tree was then constructed using the Molecular Evolutionary Genetics Analysis 7.0 (MEGA7.0) software (Kumar et al. 2016). Additionally, protein sequences from model species such as *A. thaliana* and *Oryza sativa* were included for comparative analysis. The phylogenetic tree was constructed using the neighbor-joining method, with bootstrapping of 1,000 replicates and a cut-off value of 50 % (Kumar et al. 2016).

In addition, bioinformatics tools were used to analyze the expression patterns of these genes in the following tissues: flower, flower bud, leaf, root1, root2 and stem in the *I. triloba* genome and flower_callus, stem_callus, flower, flower bud, leaf, root1, root2 and stem in the *I. trifida* genome. For both species, information was obtained from the RNA sequencing data available in the database of these species. All *GPX* gene expression levels were quantified using fragments per kilobase of exon per million fragments mapped (FPKM) values. The *GPX* gene heatmaps were drafted using the CIMMiner algorithm.

Six *GPX* genes were identified in each species (*I. triloba* and *I. trifida*) (Table 1). In *I. triloba*, the *ItbGPX* gene length ranged from 510 bp (*ItbGPX5*) to 741 bp (*ItbGPX6*), and the number of amino acids ranged from 167 (*ItbGPX4*) to 246 (*ItbGPX6*). The molecular weight in this species ranged from 18.70 kDa (*ItbGPX5*) to 27.06 kDa (*ItbGPX6*). In *I. trifida*, the *ItfGPX* gene length ranged from 507 bp (*ItfGPX4*) to 741 bp (*ItfGPX6*), and the number of amino acids from 168 (*ItfGPX4*) to 246 (*ItfGPX6*). The molecular weight in this species ranged from 18.67 kDa (*ItfGPX5*) to 26.98 kDa (*ItfGPX6*). The theoretical isoelectric point in *I. triloba* ranged from 5.70 (*ItbGPX4*) to 9.55 (*ItbGPX6*), and that in *I. trifida* from 6.12 (*ItfGPX4*) to 9.48 (*ItfGPX6*) (Table 1). The GRAVY value of all proteins was above -0.099, indicating that GPX is hydrophilic with high solubility in water (Table 1). The basic physicochemical properties of GPX and the results of the other analyses are shown in Table 1. The subcellular location of most genes was predicted to be in mitochondria and chloroplasts. The same was observed in *C. annuum* L. (Wang et al. 2023). *GPX* members may be involved in the response mechanism to abiotic stresses in these studied species.

By investigating the exon/intron structures of the *GPX* genes, it is possible to understand the organization of these genes in terms of coding and non-coding sequences in *I. triloba* and *I. trifida* (Figures 1A and 2A). This information regarding the genomic structure and expression of these genes is valuable. The sequence motifs were analyzed in two ancestral sweet potatoes. The preserved motif analysis results showed that the motifs 1, 2 and 3 were observed among all members studied in *I. triloba* and *I. trifida* (Figures 1B and 2B). All sequences were submitted to the database (Paysan-Lafosse et al. 2023), and it was observed that they presented the specific domain for GPX (IPR000889).

The chromosomal distribution of *GPX* genes showed the same pattern in both *I. trifida* and *I. triloba*. Chromosomal location analysis showed

Table 1. Physicochemical characteristics of GPX in *Ipomoea triloba* and *I. trifida*.

Gene name	*I. triloba* and *I. trifida* ID	Chromosome location	aa	Length (bp)	kDa	pI	GRAVY value	Subcellular location
ItbGPX1	itb04g13060.t1	chr04: 13068368-13064459	206	621	23.33	6.61	-0.271	Chloroplast/mitochondrion
ItbGPX2	itb04g24760.t1	chr04: 29503149-29500864	190	573	21.09	8.93	-0.099	Chloroplast/mitochondrion
ItbGPX3	itb07g05680.t1	chr07: 3892155-3888463	232	699	25.52	9.22	-0.158	Chloroplast/mitochondrion
ItbGPX4	itb08g00190.t1	chr08: 189319-187524	167	504	19.05	5.70	-0.330	Chloroplast/mitochondrion
ItbGPX5	itb08g00200.t1	chr08: 191861-189671	169	510	18.70	6.32	-0.402	Chloroplast/mitochondrion
ItbGPX6	itb09g30690.t1	chr09:31311538-31308721	246	741	27.06	9.55	-0.140	Mitochondrion
ItfGPX1	itf04g12680.t1	chr04: 9929852-9925946	206	621	23.29	6.61	-0.213	Mitochondrion
ItfGPX2	itf04g25440.t1	chr04: 26379800-26376567	174	525	19.37	8.73	-0.353	Chloroplast/mitochondrion
ItfGPX3	itf07g05610.t1	chr07: 3688362-3684689	232	699	25.57	9.30	-0.150	Chloroplast/mitochondrion
ItfGPX4	itf08g00180.t1	chr08: 81675-79845	168	507	19.23	6.12	-0.371	Chloroplast/mitochondrion
ItfGPX5	itf08g00190.t1	chr08: 84418-81974	169	510	18.67	6.32	-0.386	Chloroplast/mitochondrion
ItfGPX6	itf09g26690.t1	chr09: 22980742-22978233	246	741	26.98	9.48	-0.104	Mitochondrion

ID: identification; aa: amino acids; kDa: molecular weight; pI: isoelectric point; GRAVY: grand average of hydropathy.

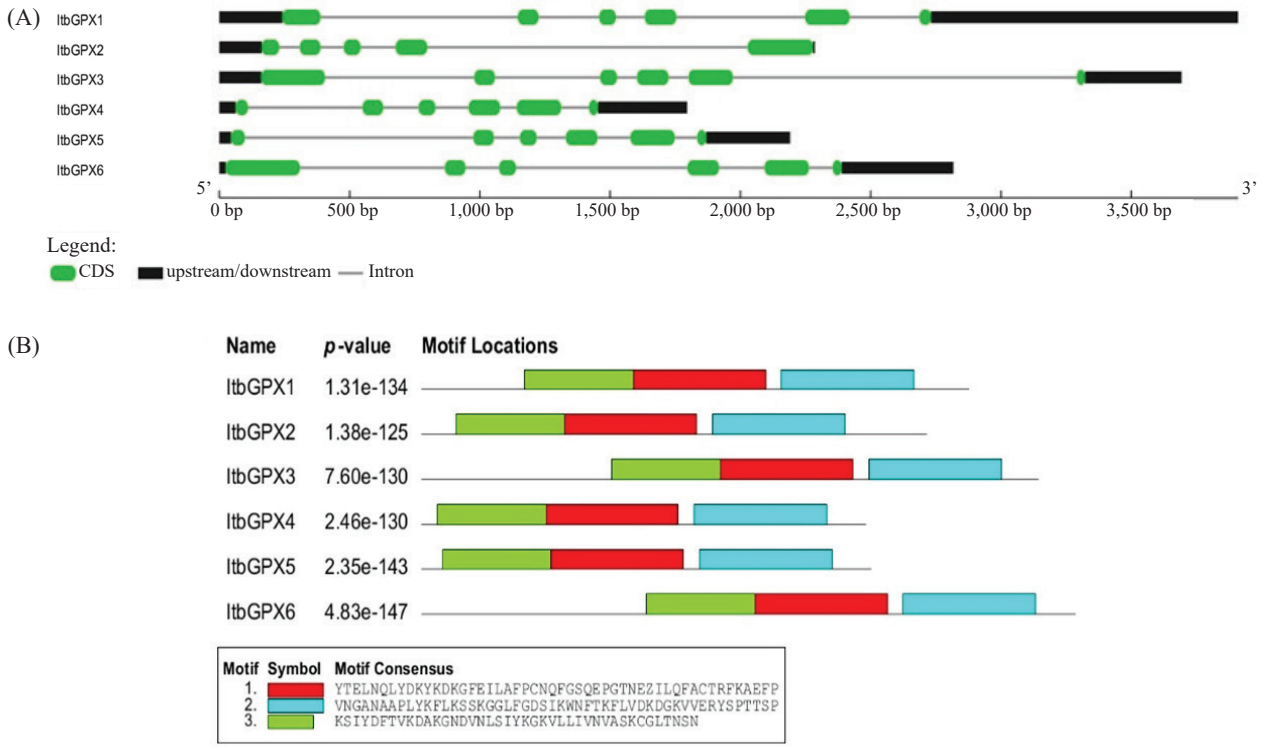

Figure 1. Exon/intron structure analysis of *GPX* and conserved motifs in *Ipomoea triloba*. A) the green boxes, gray lines and black boxes represent exons, introns and untranslated regions, respectively; B) conserved motifs in predicted GPX proteins identified by the MEME tool.

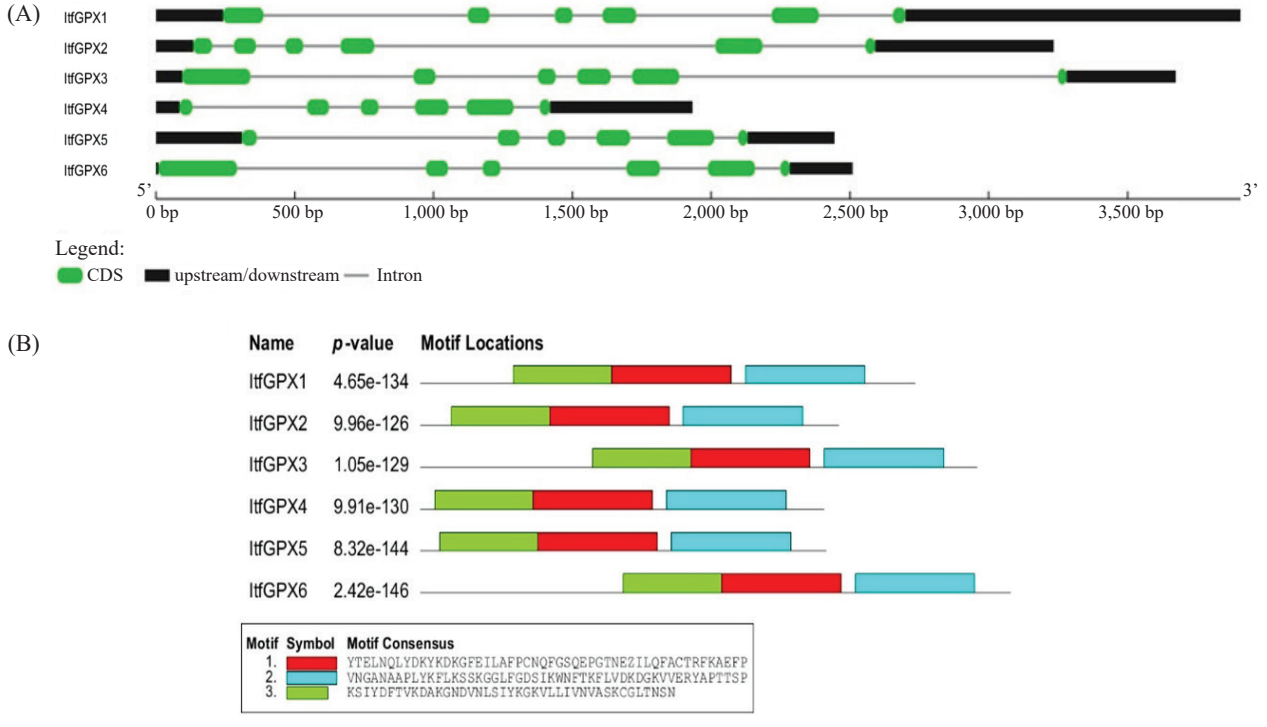

Figure 2. Exon/intron structure analysis of *GPX* and conserved motifs in *Ipomoea trifida*. A) the green boxes, gray lines and black boxes represent exons, introns and untranslated regions, respectively; B) conserved motifs in predicted GPX proteins identified by the MEME tool.

that the *GPX* genes were randomly distributed on four chromosomes in *I. trifida and I. triloba* (Figures 3A and 3B). In both *I. trifida and I. triloba*, two *GPX* genes were located on the chromosome 4, two on the chromosome 8, one on the chromosome 7 and one on the chromosome 9. Based on this chromosomal distribution, the expansion of the *GPX* genes was examined in the *I. triloba* and *I. trifida* genomes, being found four duplication pairs in *I. trifida* with a high rate of sequence similarity (Figure 3; Table 2).

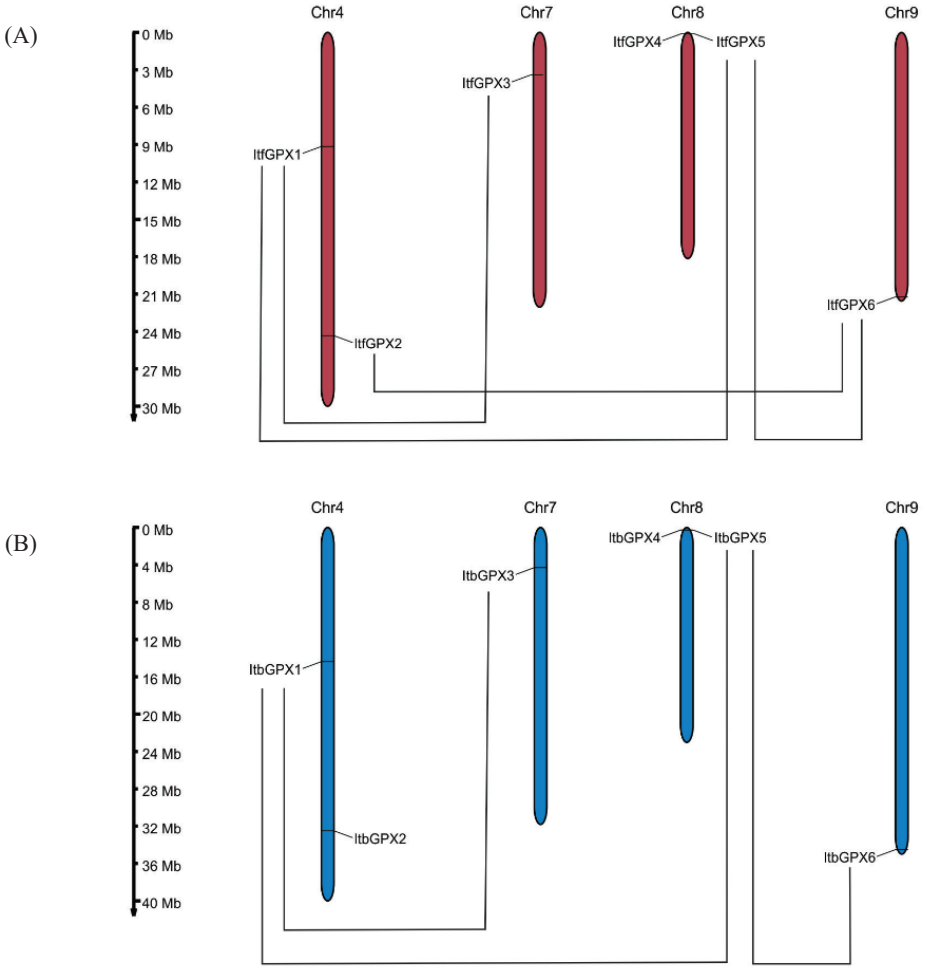

Figure 3. Chromosomal distribution and duplication events of *GPX* genes in sweet potato for *Ipomoea triloba* (A) and *I. trifida* (B). The black lines represent duplicated genes (see Table 2). The number of chromosomes and their size in Mb are indicated at the top of each bar. The vertical scale represents the size of the chromosome.

Table 2. Duplication data of paralogous gene pairs among *Ipomoea trifida* and *I. triloba GPX* genes. *Ka* is the non-synonymous substitution number per non-synonymous site, *Ks* the number of the synonymous substitution site and *Ka*/*Ks* the ratio of non-synonymous to synonymous substitutions.

Paralogous pairs	Chromosomal location	Duplication event	*Ka*	*Ks*	*Ka*/*Ks*	Purifying selection
ItrfGPX1/ItrfGPX3	Chr4/Chr7	Segmental	0.37	0.34	1.09	No
ItrfGPX1/ItrfGPX5	Chr4/Chr8	Segmental	0.39	0.30	1.30	No
ItrfGPX2/ItrfGPX6	Chr4/Chr9	Segmental	0.44	0.26	1.69	No
ItrfGPX5/ItrfGPX6	Chr8/Chr9	Segmental	0.18	0.12	1.50	No
ItlbGPX1/ItlbGPX3	Chr4/Chr7	Segmental	0.35	0.37	0.95	Yes
ItlbGPX1/ItlbGPX5	Chr4/Chr8	Segmental	0.37	0.32	1.16	No
ItlbGPX5/ItlbGPX6	Chr8/Chr9	Segmental	0.38	0.12	3.17	No

No tandem duplication was found between the *GPX* genes in the two studied species; only segmental duplications were found (four and three for *I. trifida* and *I. triloba*, respectively). The findings of the present study suggest that *GPX* genes may have arisen through gene duplication, with segmental duplication emerging as a predominant driving force for expansion within the investigated species. Similar studies have focused on different gene families in sweet potato (Liu et al. 2023, Zhang et al. 2023). The duplication process is implicated in augmenting functional divergence, a pivotal factor facilitating adaptation to dynamic climatic changes (Conant & Wolfe 2008).

The direction and magnitude of pressure selection can be inferred based on the *Ka*/*Ks* ratio, where *Ka*/*Ks* > 1 indicates a positive selection, *Ka*/*Ks* = 1 a neutral evolution and *Ka*/*Ks* < 1 a purifying selection (Ali et al. 2017). To detect the selection pressure acting on *GPX* genes, the *Ka*, *Ks* and *Ka*/*Ks* values were analyzed in the two *Ipomea* species (Table 2). In *I. triloba*, only one pair of one gene (*ItlbGPX1*/*ItlbGPX3*) had a *Ka*/*Ks* ratio of < 1, indicating that these genes evolved through purifying selection. The *Ka*/*Ks* ratios of all four *GPX* gene pairs in *I. tripod* and two gene pairs in *I. triloba* were > 1. Thus, a positive selection (Darwinian selection) may have resulted in the accumulation of progressive mutations and spread them throughout the population (Si et al. 2022).

To elucidate the evolutionary relationship of GPX proteins in *I. triloba* and *I. trifida*, a phylogenetic tree was constructed using four species (*I. triloba*, *I. trifida*, *A. thaliana* and *O. sativa*) (Figure 4). All GPX proteins from *I. triloba* and *I. trifida* were unequally distributed across the branches of the phylogenetic tree (Figure 4). According to these results, the clades were named Group I, II, III and IV, respectively (Figure 4). The findings indicated that the number and type of GPX protein in each sweet potato group may differ from those in its two diploid relatives. The data regarding the number of groups corroborate those reported by Wang et al. (2023).

The presence of a homolog of each GPX protein from *I. triloba* and *I. trifida* in the *A. thaliana* groups suggests a possible evolutionary conservation of these genes between the two studied species. The similarity between the *GPX* genes of these sweet potato ancestors and *A. thaliana*, for example, may indicate similar functions of these proteins, in terms of the antioxidant response and protection against oxidative damage within the cell. Furthermore, the recent divergence between the genomes of *I. triloba* and *I. trifida* may be responsible for the high sequence identity between the *GPX* genes of these two species. According to Wu et al. (2018), a whole-genome triplication event occurred in an ancient *Ipomoea* lineage ancestor approximately 46.1 million years ago (Mya). This event occurred before both the approximately 3.6 Mya divergence of *I. nil* from the lineage that includes *I. trifida* and *I. triloba* and the approximately 2.2 Mya divergence between *I. trifida* and *I. triloba* (Wu et al. 2018).

To better understand the behavior of these genes, RNA sequencing data (FPKM values) were obtained from the *I. trifida* and *I. triloba* genomes from the database. As shown in Figure 5, the *ItfGPX5* gene in *I. trifida* had a higher expression profile than the other genes (Figure 5A). Other genes were also expressed (Figure 5A). The *ItbGPX5* gene was highly expressed in all studied tissues. The *ItbGPX3* gene was expressed in the flower bud, leaf and stem tissues. However, mild expression levels were also observed in other tissues (Figure 5B). With respect

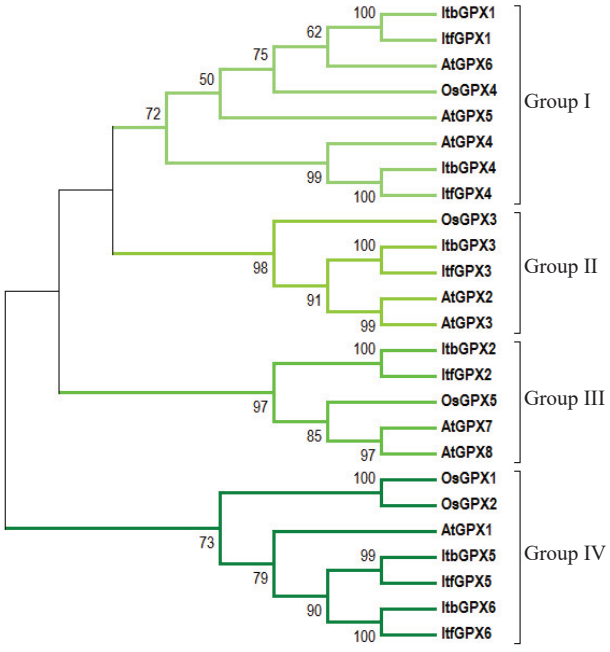

Figure 4. Phylogenetic tree of GPX protein sequences from *Ipomoea triloba*, *I. trifida*, *Arabidopsis thaliana* and *Oryza sativa*. A neighbor-joining phylogenetic tree was constructed using the MEGA7.0 software with 1,000 bootstrap replicates. Groups I, II, III and IV are indicated by square brackets.

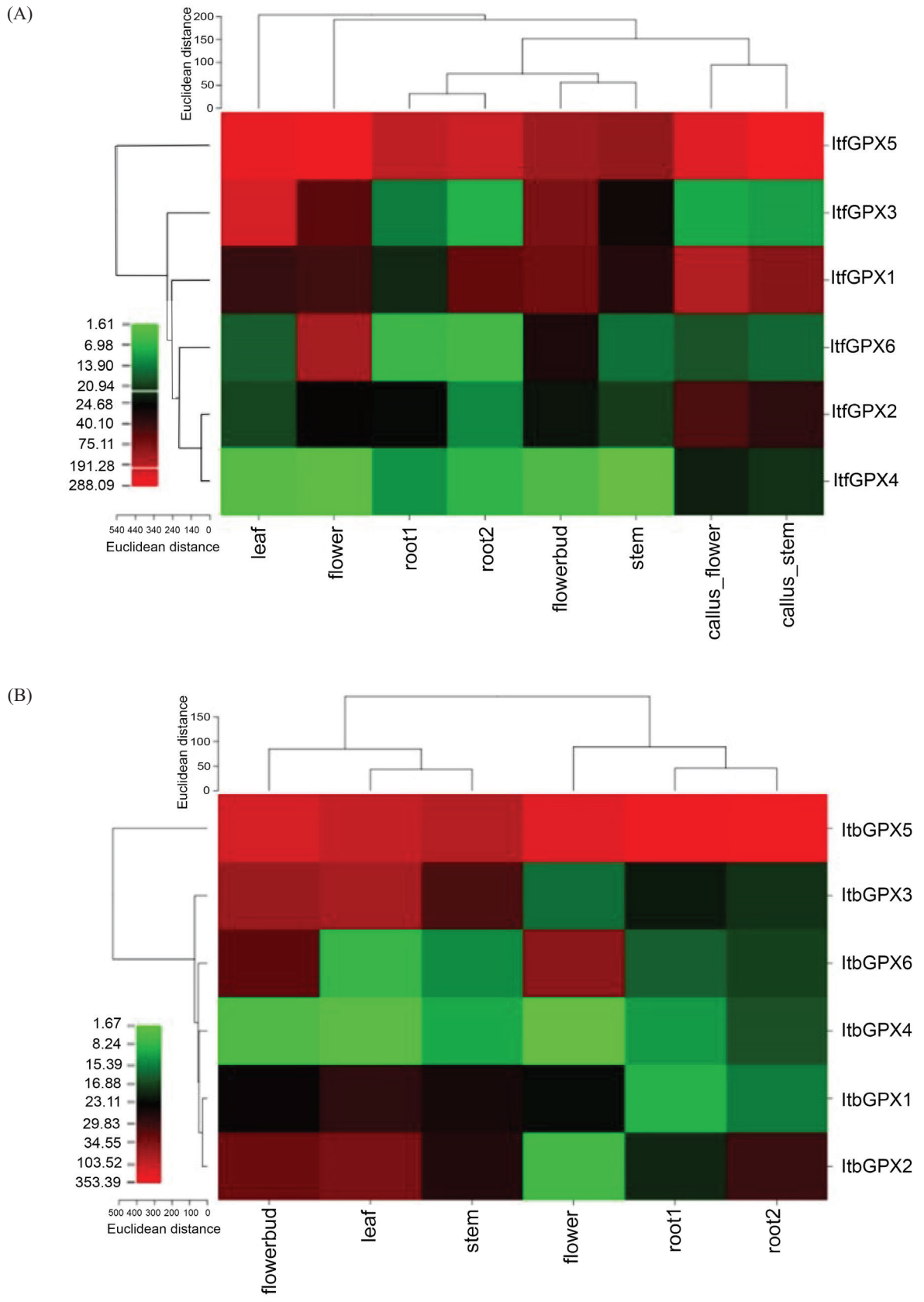

Figure 5. *In silico* expression profiles for *Ipomoea trifida* (A) and *I. triloba* (B). The color bar represents the fragments per kilobase of exon per million fragments mapped (FPKM) value obtained in the genome.

to the biological mechanism, it is believed that genes can be expressed according to the type of stress and specific tissue.

These results represent a preliminary exploration of *GPX* genes and facilitate future investigation on the biological functions of GPX proteins in sweet potato.

Six *ItfGPX* (*Ipomoea trifida*) and six *ItbGPX* (*I. triloba*) genes were identified and characterized in this study.

The phylogenetic analysis showed that the *GPX* genes of *I. trifida*, *I. triloba*, *A. thaliana* and *O. sativa* are classified into four groups;

The *GPX* genes in *I. trifida* and *I. triloba* were highly conserved, when compared with those in *A. thaliana* and *O. sativa*;

The expression patterns in various tissues and development stages may involve different plant growth and development processes.

REFERENCES

ALAM, M. K. A comprehensive review of sweet potato (*Ipomoea batatas* [L.] Lam): revisiting the associated health benefits. *Trends in Food Science and Technology*, v. 115, n. 3, p. 512-529, 2021.

ALI, H.; LIU, Y.; AZAM, S. M.; PRIYADARSHANI, S. V. G. N.; LI, W.; HUANG, X.; QIN, Y. Genomic survey, characterization, and expression profile analysis of the SBP genes in pineapple (*Ananas comosus* L.). *International Journal of Genomics*, v. 2017, e1032846, 2017.

ALTSCHUL, S. F.; MADDEN, T. L.; SCHAFFER, A. A.; ZHANG, J.; ZHANG, Z.; MILLER, W.; LIPMAN, D. J. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, v. 25, n. 17, p. 3389-3402, 1997.

BAILEY, T. L.; BODEN, M.; BUSKE, F. A.; FRITH, M.; GRANT, C. E.; CLEMENTI, L.; REN, J.; LI, W. W.; NOBLE, W. S. MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Research*, v. 37, suppl. 2, p. W202-W208, 2009.

BELA, K.; HORVÁTH, E.; GALLÉ, Á.; SZABADOS, L.; TARI, I.; CSISZÁR, J. Plant glutathione peroxidases: emerging role of the antioxidant enzymes in plant development and stress responses. *Journal of Plant Physiology*, v. 176, n. 1, p. 192-201, 2015.

BELA, K.; RIYAZUDDIN, R.; HORVÁTH, E.; HURTON, Á.; GALLÉ, Á.; TAKÁCS, Z.; CSISZÁR, J. Comprehensive analysis of antioxidant mechanisms in *Arabidopsis* glutathione peroxidase-like mutants under salt- and osmotic stress reveals organ-specific significance of the AtGPXL's activities. *Environmental and Experimental Botany*, v. 150, n. 1, p. 127-140, 2018.

BHAT, J. A.; DESHMUKH, R.; ZHAO, T.; PATIL, G.; DEOKAR, A.; SHINDE, S.; CHAUDHARY, J. Harnessing high-throughput phenotyping and genotyping for enhanced drought tolerance in crop plants. *Journal of Biotechnology*, v. 324, n. 1, p. 248-260, 2020.

CHEN, M.; LI, K.; LI, H.; SONG, C. P.; MIAO, Y. The glutathione peroxidase gene family in *Gossypium hirsutum*: genome-wide identification, classification, gene expression and functional analysis. *Scientific Reports*, v. 7, n. 1, e44743, 2017.

CHOU, K. C.; SHEN, H. B. Plant-mPLoc: a top-down strategy to augment the power for predicting plant protein subcellular localization. *PLoS One*, v. 5, n. 6, e11335, 2010.

CONANT, G. C.; WOLFE, K. H. Probabilistic cross-species inference of orthologous genomic regions created by whole-genome duplication in yeast. *Genetics*, v. 179, n. 3, p. 1681-1692, 2008.

GAO, F.; CHEN, J.; MA, T.; LI, H.; WANG, N.; LI, Z.; ZHANG, Z.; ZHOU, Y. The glutathione peroxidase gene family in *Thellungiella salsuginea*: genome-wide identification, classification, and gene and protein expression analysis under stress conditions. *International Journal of Molecular Sciences*, v. 15, n. 2, p. 3319-3335, 2014.

HERBETTE, S.; ROECKEL-DREVET, P.; DREVET, J. R. Seleno-independent glutathione peroxidases: more than simple antioxidant scavengers. *FEBS Journal*, v. 274, n. 9, p. 2163-2180, 2007.

HU, B.; JIN, J.; GUO, A. Y.; ZHANG, H.; LUO, J.; GAO, G. GSDS 2.0: an upgraded gene feature visualization server. *Bioinformatics*, v. 31, n. 8, p. 1296-1297, 2015.

ISLAM, T.; MANNA, M.; KAUL, T.; PANDEY, S.; REDDY, C. S.; REDDY, M. K. Genome-wide dissection of *Arabidopsis* and rice for the identification and expression analysis of glutathione peroxidases reveals their stress-specific and overlapping response patterns. *Plant Molecular Biology Reporter*, v. 33, n. 5, p. 1413-1427, 2015.

JANA, G. A.; YAISH, M. W. Genome-wide identification and functional characterization of glutathione peroxidase genes in date palm (*Phoenix dactylifera* L.) under stress conditions. *Plant Gene*, v. 23, e100237, 2020.

KUMAR, S.; STECHER, G.; TAMURA, K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, v. 33, n. 7, p. 1870-1874, 2016.

LI, W.; HUAI, X.; LI, P.; RAZA, A.; MUBARIK, M. S.; HABIB, M.; KHAN, R. S. A. Genome-wide characterization of glutathione peroxidase (GPX) gene family in rapeseed (*Brassica napus* L.) revealed their role in multiple abiotic stress response and hormone signaling. *Antioxidants*, v. 10, n. 9, e1481, 2021.

LIU, E.; LI, Z.; LUO, Z.; XU, L.; JIN, P.; JI, S.; ZHOU, G.; WANG, Z.; ZHOU, Z.; ZHANG, H. Genome-wide identification of DUF668 gene family and expression analysis under drought and salt stresses in sweet potato [*Ipomoea batatas* (L.) Lam]. *Genes*, v. 14, n. 1, e217, 2023.

LIU, Q. Improvement for agronomically important traits by gene engineering in sweet potato. *Breeding Science*, v. 67, n. 1, p. 15-26, 2017.

MARGIS, R.; DUNAND, C.; TEIXEIRA, F. K.; MARGIS-PINHEIRO, M. Glutathione peroxidase family: an evolutionary overview. *FEBS Journal*, v. 275, n. 15, p. 3959-3970, 2008.

MEITHA, K.; PRAMESTI, Y.; SUHANDONO, S. Reactive oxygen species and antioxidants in postharvest vegetables and fruits. *International Journal of Food Science*, v. 2020, e8817778, 2020.

PARVEEN, K.; SADDIQUE, M. A. B.; ALI, Z.; REHMAN, S. U.; KHAN, Z.; WAQAS, M.; MUNEER, M. A. Genome-wide analysis of glutathione peroxidase (GPX) gene family in chickpea (*Cicer arietinum* L.) under salinity stress. *Gene*, v. 898, e148088, 2024.

PASSAIA, G.; MARGIS-PINHEIRO, M. Glutathione peroxidases as redox sensor proteins in plant cells. *Plant Science*, v. 234, n. 1, p. 22-26, 2015.

PAYSAN-LAFOSSE, T.; BLUM, M.; CHUGURANSKY, S.; GREGO, T.; PINTO, B. L.; SALAZAR, G. A.; BATEMAN, A. InterPro in 2022. *Nucleic Acids Research*, v. 51, n. D1, p. D418-D427, 2023.

SANTOS, T. B.; RIBAS, A. F.; SOUZA, S. G. H. de; BUDZINSKI, I. G. F.; DOMINGUES, D. S. Physiological responses to drought, salinity, and heat stress in plants: a review. *Stresses*, v. 2, n. 1, p. 113-135, 2022.

SI, Z.; WANG, L.; QIAO, Y.; ROYCHOWDHURY, R.; JI, Z.; ZHANG, K.; HAN, J. Genome-wide comparative analysis of the nucleotide-binding site-encoding genes in four *Ipomoea* species. *Frontiers in Plant Science*, v. 13, e960723, 2022.

THOMPSON, J. D.; HIGGINS, D. G.; GIBSON, T. J. CLUSTAL, W. Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, v. 22, n. 22, p. 4673-4680, 1994.

VOORRIPS, R. MapChart: software for the graphical presentation of linkage maps and QTLs. *Journal of Heredity*, v. 93, n. 1, p. 77-78, 2002.

WANG, W.; CHENG, Y.; RUAN, M.; YE, Q.; YAO, Z.; WANG, R.; WAN, H. Comprehensive identification of glutathione peroxidase (GPX) gene family in response to abiotic stress in pepper (*Capsicum annuum* L.). *Gene*, v. 881, e147625, 2023.

WANG, W.; QIU, X.; YANG, Y.; KIM, H. S.; JIA, X.; YU, H.; KWAK, S. S. Sweetpotato bZIP transcription factor IbABF4 confers tolerance to multiple abiotic stresses. *Frontiers in Plant Science*, v. 10, e630, 2019.

WANG, X.; LIU, X.; AN, Y.; ZHANG, H.; MENG, D.; JIN, Y.; HUO, H.; YU, L.; ZHANG, J. Identification of glutathione peroxidase gene family in *Ricinus communis* and functional characterization of RcGPX4 in cold tolerance. *Frontiers in Plant Science*, v. 12, e707127, 2021.

WANG, Y.; CAO, S.; SUI, X.; WANG, J.; GENG, Y.; GAO, F.; ZHOU, Y. Genome-wide characterization, evolution, and expression analysis of the ascorbate peroxidase and glutathione peroxidase gene families in response to cold and osmotic stress in *Ammopiptanthus nanus*. *Journal of Plant Growth Regulation*, v. 42, n. 8, p. 502-522, 2022.

WASZCZAK, C.; CARMODY, M.; KANGASJÄRVI, J. Reactive oxygen species in plant signaling. *Annual Review of Plant Biology*, v. 69, n. 1, p. 209-236, 2018.

WU, S.; LAU, K. H.; CAO, Q.; HAMILTON, J. P.; SUN, H.; ZHOU, C.; ESERMAN, L.; GEMENET, D. C.; OLUKOLU, B. A.; WANG, H. Y.; CRISOVAN, E.; GODDEN, G. T.; JIAO, J.; WANG, X.; MERCY, K.; MANRIQUE-CARPINTERO, N.; VAILLANCOURT, B.; WIEGERT-RININGER, K.; YANG, X. S.; BAO, K.; SCHAFF, J.; KREUZE, J.; GRUNEBERG, W.; KHAN, A.; GHISLAIN, M.; MA, D. F.; JIANG, J. M.; MWANGA, R.; LEEBENS-MACK, J.; COIN, L.; YENCHO, C.; ROBIN, B. R.; FEI, Z. Genome sequences of two diploid wild relatives of cultivated sweet potato reveal targets for genetic improvement. *Nature Communications*, v. 9, n. 1, e4580, 2018.

XUE, Y.; CHEN, L.; LI, B.; XIAO, J.; WANG, H.; DONG, C.; XU, P. Genome-wide mining of *GPX* gene family provides new insights into cadmium stress responses in common carp (*Cyprinus carpio*). *Gene*, v. 821, e146291, 2022.

ZAFAR, S. A.; HAMEED, A.; ASHRAF, M.; KHAN, A. S.; QAMAR, Z. U.; LI, X.; SIDDIQUE, K. H. M. Agronomic, physiological and molecular characterisation of rice mutants revealed the key role of reactive oxygen species and catalase in high-temperature stress tolerance. *Functional Plant Biology*, v. 47, n. 5, p. 440-453, 2020.

ZHANG, J. Z.; HE, P. W.; XU, X. M.; LÜ, Z. F.; CUI, P.; GEORGE, M. S.; LU, G. Q. Genome-wide identification and expression analysis of the xyloglucan endotransglucosylase/hydrolase gene family in sweet potato [*Ipomoea batatas* (L.) Lam]. *International Journal of Molecular Sciences*, v. 24, n. 1, e775, 2023.

ZHANG, Z.; LI, J.; ZHAO, X. Q.; WANG, J.; WONG, G. K.; YU, J. KaKs_Calculator: calculating Ka and Ks through model selection and model averaging. *Genomics, Proteomics & Bioinfomatics*, v. 4, n. 4, p. 259-263, 2006.

ZHOU, Y.; LI, J.; WANG, J.; YANG, W.; YANG, Y. Identification and characterization of the glutathione peroxidase (GPX) gene family in watermelon and its expression under various abiotic stresses. *Agronomy*, v. 8, n. 10, e206, 2018.