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IDENTIFICATION AND HEAVY METALS-INDUCED EXPRESSION ANALYSIS OF THE OLIGOPEPTIDE TRANSPORTERS (OPT) GENE FAMILY IN TOMATO

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Abstract

The oligopeptide transporters are membrane integral proteins playing pivotal role in translocating secondary amino acids, small peptides, organic nitrogen mobilization and contribute in variety of biological activities. In this study, we have performed comprehensive bioinformatic identification of tomato OPT gene family and a total of 16 OPT genes were identified in tomato. These proteins were classified into two subfamilies including PT sub-family and YSL sub-family with eight genes in each based on phylogenetic analysis. Moreover, OPT exhibited a unique gene configuration that was consistent with previously reported studies and validate its phylogenetic classification. The putative OPT gene family members exhibited diverse expression pattern and few genes express in specific tissues. For example, SlYSL1, SlOPT3, and SlOPT5 expressed in flowers and SlOPT1, SlOPT2, and SlYSL2 in roots. However, some genes showed high expression in fruits at various developmental stages. These include SlOPT6, SlOPT8, SlYSL3, SlYSL4, and SlYSL5. It was observed that expression of some SlOPTs were induced under heavy metals such as calcium, cadmium, iron, magnesium, potassium, and lead in both root and shoot. This suggested a potential involvement of these genes in tomato adaptation under stress. In summary, our work is the first comprehensive analysis of oligopeptide transporters in tomato and an important resource for prioritizing genes especially in metal ions mobilizing and nutrient deficiency stress adaptation.

Key words: Tomato, Oligopeptide, Heavy metal, Induced, Expression analysis.

Introduction

Organisms transport broad range of substrates either organic or inorganic across plasma membrane from the extracellular environment to cell as a source of nutrients. Micronutrients such as heavy metals (either essential or non-essential) are vital for various physiological and biological processes in plants such as chlorophyll biosynthesis, nitrogen assimilation, photosynthesis, and respiration (Morrissey & Guerinot, 2009). The heavy metals pollution is one of the negative effects of industrialization, which is dexterous not only to human health but also to environment. However, excess amount of heavy metal uptake causes cellular toxicity by inhibiting protein binding activity or enzyme functions through binding with sulfhydryl groups of protein, disturbance in cellular transportation system, and oxidative damages (Williams & Miller, 2001). Therefore, plants have evolved various mechanism on heavy metal ion toxicity tolerance and metal scavenging systems (Li *et al.*, 2015). In past few decades, molecular biology techniques have enabled in identification and characterization of different metal transportation gene families (Williams & Miller, 2001).

In plants, different substrate specific transporters are localized in plasma membrane and are associated with active transportation of sucrose, peptides, amino acids, and metals (Koh *et al.*, 2002). The peptide transporters are proteins that transport peptides into cell as source of amino acids for protein biosynthesis or as a nitrogen and carbon (Lubkowitz *et al.*, 1997). The peptide transporters are divided into various classes such as the oligopeptide transporters (OPT), nitrate transporters (NRT), and the ABC-type transporter family (Rentsch *et al.*, 2007; Zhao *et al.*, 2010). The membrane proteins, OPT, can transport a wide range of substrate across membrane and play pivotal role in various biological processes. The OPT proteins are first characterized in yeast (Lubkowitz *et al.*, 1997) and later in bacteria (Lubkowitz *et al.*, 1998) and plants (Koh *et al.*, 2002).

The OPT proteins are multifunctional proteins and may involve in four biological processes; (i) nitrogen assimilation or mobilization (Koh *et al.*, 2002; Pike *et al.*, 2009), (ii) long distance metal transportation (Stacey *et al.*, 2008), (iii) glutathione transporters (Zhang *et al.*, 2004), (iv) heavy metal sequestration (Cagnac *et al.*, 2004). The plants OPT proteins are classified into two sub families based on phylogeny; the peptide transporter (PT) sub-family and yellow stripe-like (YSL) sub-family (Curie *et al.*, 2001). The PT sub-family contained 18 or 11-14 amino acid highly conserved NPG (Asn-Pro-Gly) or KIPPR (Lys-Ile-Pro-Pro-Arg) motifs and considered to transport tetrapeptide or pentapeptides (Koh *et al.*, 2002; Osawa *et al.*, 2006), while YSL lack these motifs and function in metal transport (Inoue *et al.*, 2009; Lee *et al.*, 2009).

The PT subfamily proteins has been identified and characterized in *Arabidopsis*, *Brassica*, and rice (Bogs *et al.*, 2003; Osawa *et al.*, 2006; Lee *et al.*, 2009) and have been involved in metal detoxification (Bogs *et al.*, 2003), seeds germination (Müntz, 1998), embryo development (Stacey *et al.*, 2008), nitrogen transportation (Williams and Miller, 2001), and glutathione transporter (Bogs *et al.*, 2003; Zhang *et al.*, 2004). The YSL subfamily is considered to function in metal transportation such as Mg, Fe, Cd and so on (Inoue *et al.*, 2009; Lee *et al.*, 2009) and multiple YSL transporter genes have been identified from *Brachypodium distachyon* (Yordem *et al.*, 2011), *Arabidopsis thaliana* (Gross, 2003), *Zea mays* (Yordem *et al.*, 2011), and *Oryza sativa* (Koike *et al.*, 2004). In maize, ZmYSL1 demonstrated as Phyto siderophore

transporter (Curie *et al.*, 2001; Roberts *et al.*, 2004). In rice, various YSL (OsYSL2, OsYSL15, OsYSL18) are orthologs to ZmYSL1, in that they facilitate to transport metal-nicotianamine (Koike *et al.*, 2004; Aoyama *et al.*, 2009; Inoue *et al.*, 2009; Lee *et al.*, 2009). Moreover, some OPT members of rice (OsOPT1, OsOPT3, OsOPT4, OsOPT5, OsOPT7) have ability to transport nicotianmine bound iron (Vasconcelos *et al.*, 2008).

To understand the potential role of OPT gene family, it is important to identify OPT gene family members in tomato genome. In this study, we are presenting identification of OPT gene family in tomato, including chromosome distribution, gene structure analysis, motif analysis, cis regulatory elements prediction, *in silico* subcellular location prediction, and phylogenetic analysis. In addition, the tissue/organ specific expression profile analysis under normal conditions as well as under various heavy metal stresses were performed. The systematic analysis of OPT gene family in tomato can lay a foundation for future functional studies in tomato as well as other plant species.

Material and Methods

Identification of OPT gene family in tomato: Peptide sequences of Arabidopsis OPT genes family (Koh *et al.*, 2002) extracted from the The Arabidopsis Information Resource (TAIR) (Reiser and Rhee, 2005) were used as queries in searches with default parameters against the SOL genome network for tomato (Fernandez-Pozo *et al.*, 2015). The protein sequences of tomato genome were downloaded from SOL genome. The OPT domain (PF03169) pattern was retrieved from the Pfam database (Finn *et al.*, 2008). The candidate OPT genes were search using HMMER 3.0 with a default setting and all redundant OPT sequences were excluded. The deduced OPT sequences were further subjected for OPT domain by using NCBI considered domain searched (Marchler-Bauer *et al.*, 2017) and Simple Modular Architecture Research Tool (SMART) (Schultz *et al.*, 1998). The tomato OPT genes were named according to their orders on the chromosomes. Moreover, physicochemical properties of OPT proteins, including the isoelectric point (pI), the grand average of hydropathy (GRAVY), molecular weight (kDa) of each OPT protein were assessed using Sequence Manipulation Suite (Stothard, 2000).

Multiple sequence alignment (MSA) and phylogeny: The multiple sequence alignment of tomato OPT genes were performed using Clustal Omega program (Sievers *et al.*, 2011). To evaluate the classification of OPT proteins in tomato, a phylogenetic tree was constructed using OPT protein sequences from Arabidopsis (Koh *et al.*, 2002) and rice genome (Vasconcelos *et al.*, 2008). MEGAX program (Kumar *et al.*, 2018) was used to construct an unrooted neighbour joining (NJJ) (Saitou and Nei, 1987) tree with bootstrap set at 1000 replicates, Jones-Taylor-Thornton (JTT) model, and pairwise deletion option.

Chromosome location, cis-regulatory elements, conserved motifs, and gene structure analysis: The chromosome location of OPT genes was obtained from SOL genome database (Fernandez-Pozo *et al.*, 2015). The MAP2Chromomse program V2 was used to draw position of each protein on chromosome. The tomato OPT genes genome and CDS sequences were downloaded from SOL genome and submitted to Gene Structure Display Server 2.0 (Hu *et al.*, 2015) to illustrate gene structure integrity (exon/intron). Tomato OPT protein sequences were further submitted to MEME suite for conserved motif prediction. MEME suite (Bailey & Elkan, 1994) was set with following parameter i) maximum number of motifs - 10, (ii) number of repetitions – any, (iii) optimum motif width set to $\geq$ 10 and $\leq$ 50. The 1000bp promotor sequences of each OPT gene was downloaded from SOL genome and queried to PlantCARE program (Lescot *et al.*, 2002) for cis-regulatory motif prediction. Moreover, In-silico subcellular location of each OPT gene were predicted in WoLF PSORT (Horton *et al*., 2007) by submitting their protein sequences.

Plant growth condition, material collection, and heavy metal stress: The tomato cv. Micro-Tom seeds were surface sterilized and sown in green house under conditions; 12 h (light/dark) photoperiod at 25 °C, 300 µmol/m^2/s, and 80% relative humidity. The plant parts like roots, leaves, flower (flower bud and fully opened) were harvested from 4-week-old seedling while fruit tissues were harvested from 1 cm, 2 cm, 3 cm, mature green, breaker, and breaker plus 10 days fruits. All the samples were collected from three independent plants mixed thoroughly.

To demonstrate the heavy metal induced gene expression in tomato OPT gene family, four-week-old tomato seedling were exposed to 750 µM $Pb(NO_3)_2$, 100 µM $CdCl_2$, 20 mM $FeCl_3$, 500 µM $CaCl_2$, 100 µM KH_2PO_4, and 100 µM $MgSO_4$. The control plants were treated with fresh water. Shoots including stem and leaves, and roots were harvest after 0h, 6h, 12h, and 24h after treatment. Three independent biological replicates were collected, and six seedlings were used for each treatment. All the samples were immediately frozen in liquid nitrogen and were stored at −80 °C till further analysis.

Nucleic acid extraction, cDNA preparation and qRT-PCR analysis: To verify the expression of tomato COI genes, total RNA was extracted from all samples using TRIZOL reagent following manufacturer's instructions. The RNA was qualified using nanodrop (Thermo USA) and quality was assessed by 2% (w/v) stained agarose gel electrophoresis. The cDNA was synthesised using Prime Script ™ RT reagent Kit with gDNA Eraser (Takara, JAPAN) and qRT-PCR performed using SYBR-Premix Ex Taq-II (TliRNaseH Plus) on CFX96 Touch ™ Real-Time PCR Detection System (BIO-RAD, USA). Th gene specific primer pairs used for qPCR are listed in Table 1. The *SlUBQ* (*Solyc01g056940*) used as housekeeping gene. The $2^{-\Delta\Delta Ct}$ method was used to calculate relative expression (Livak and Schmittgen, 2001). The heat map was generated using MeV 4.9 software package.

Table 1. List of qPCR primers used in this study.

Gene name	Primer forward	Primer reverse
SlOPT1	ACCCTTACATCCCCGCGTTCC	TGGTGCCGGGTATGACCCTT
SlOPT2	TGCGGATGAGAGCCCGAGCA	GGACGAGCACCAACTGGCAGG
SlOPT3	ACCGTCCCCATCACCGACGA	GGCCTAACGGAACCACCGCA
SlOPT4	TGGTGCTGTTGCGTCTGGGT	GGTGCGCACAGAAGGCCCAA
SlOPT5	AGTCCTACCAGCCTTCACGGCA	CCTCTGCAGGGGATGCACGC
SlOPT6	ACGATTTTCGCGAACTCCGGC	AAGCCCGGCCCATCCAAAGC
SlOPT7	GAGGGCACGAGTTGGGCGTT	AAACAGTTCCAGCGCCCGCA
SlOPT8	AGTTCAGCGATTCCGAGCCTCA	GCCCCCACCACGAAAGCTGA
SlYSL1	TCAACATGGCAACAGCGTGGT	GGGCCATCGCGCCTCCTAAA
SlYSL2	CCTTGGACTTGCCCCGGTGA	CGCTCCAAGAAGCACGGGGA
SlYSL3	CAGCAGCCATGTGGTGGCCT	AGCCCCTTTAGGCCCGAACC
SlYSL4	TCCATCGCGCTCCTCCTTGGT	CTGCCTCACCCCTGCTGCTG
SlYSL5	ACTGGGCAGCAGCTGTGGGA	GCCCAGCAATCACACCACCGT
SlYSL6	GGTGCATTGCTACCGGTGCCT	GTTGCTGGTGGCATTCCCGC
SlYSL7	GCTCCGTCCAACCACCACCG	GTCCCAGAAACCATGCCCGGA
SlYSL8	CTTATGGAGTTGGCTTTGCTG	TTCCCAAATACACAAACCCTGC
SlUBI	CACCAAGCCAAAGAAGATCA	TCAGCATTAGGGCACTCCTT

Results

Identification of SlOPT genes in tomato genome: By removing redundant sequences, we obtained sixteen candidates OPT genes in tomato genome. The OPT genes were named according to their chronological position on chromosomes and classified into two sub-families including SlOPT and SlYSL according to their phylogeny and sequence similarity with Arabidopsis AtOPTs. The characteristic features of OPT genes are given in Table 2. *In-silico* subcellular prediction analysis of tomato OPT proteins were performed to explore putative functions of candidate genes. All the candidate OPT proteins are localized in membranes of various cellular organelles such as endoplasmic reticulum, vascular membrane, or cytosol (Table 2). Moreover, the molecular weight (kDa) of PT sub-family proteins ranged from 82.72 (*SlOPT1*) to 84.45 (*SlOPT7*) with pI from 6.68 (*SlOPT7*) to 9.2 (*SlOPT8*). The tomato genome encodes highly hydrophobic polypeptides (0.353 (*SlOPT6*) to 0.515 (*SlOPT4*)) ranging in peptide length from 733 (*SlOPT1*) to 756 (*SlOPT6*). For YSL sub-family, the OPT genes in this clade are slightly alkaline and pI values ranged from 5.82 (*SlYSL3*) to 9.44 (*SlYSL4*). Similarly, *SlYSL* proteins molecular weight (kDa) to hydrophobicity varies from 64.19 (*SlYSL6*) to 76.17 (*SlYSL2*) and 0.425 (*SlYSL8*) to 0.551 (*SlYSL3*).

Multiple sequence alignment and phylogeny: Multiple sequence alignment of tomato OPT revealed the presence of peptide transporter (PT) motifs including NPG and KIPPR motifs (Fig. 1) which is characteristic feature of PT sub-family but absent in YSL clade. In our study, the dendrogram of tomato OPT proteins divided into two clades; the PT sub-family and YSL sub-family (Fig. 2a) that validated the multiple protein alignment of OPT protein. To further validate our phylogenetic dissection of OPT genes in tomato genome. We have investigated the phylogenetic relationship of tomato OPT proteins with rice and Arabidopsis OPT proteins. The comparison supported our phylogenetic classification of tomato SlOPT genes (Fig. 2b). The phylogenetic tree unveiled the genetic independence between PT and YSL clades.

Chromosome location and gene configuration: Tomato OPT genes were distributed on five chromosomes (Fig. 3a). For PT sub-family single SlOPT gene (SlOPT1, SlOPT7, SlOPT8) was localized on chromosome 2, 8, and 11. SlOPT2 and SlOPT3 was localized on chromosome 3 but SlOPT4, SlOPT5 and SlOPT6 was localized on chromosome 4. Similarly, for YSL clade, SlYSL6, SlYSL7, and SlYSL8 was anchored on chromosome 5, 8, and 9, respectively. Moreover, three SlYSLs (SlYSL1, SlYSL2, SlYSL3) and two SlYSLs (SlYSL4, SlYSL5) were localized on chromosome 2 and 3, accordingly (Fig. 3a). To obtain more information about SlOPT genomic organization, we have assessed gene exon and intron boundaries. We have found that most of SlOPT genes have similar intron-exon boundaries (Fig. 3c). In YSL sub-family, genes SlYSL3, SlYSL5, and SlYSL7 contain seven exons and six introns but the length of second to sixth exon was identical. Similarly, for SlYSL3 and SlYSL2, exon length in second and fifth was identical. For PT sub-family, SlOPT8, SlOPT4, SlOPT5 and SlOPT6 have five intron and six exons but the length of first exon (SlOPT8, SlOPT4) and third (SlOPT5, SlOPT6) was non-identical. In addition, a 98bp exon was found in both PT and YSL sub-family members except SlOPT2, SlOPT1, SlYSL6, SlYSL8 (Fig. 3c).

Analysis of conserved motifs and cis-regulatory elements in putative SlOPT genes: To investigate the SlOPT protein architecture, ten motifs in the peptide sequences were predicted using MEME searches. It was observed that some motifs highly conserved and found only in specific clade of OPT proteins. For an instance, motif 3, motif 5, and motif 9 were confined to PT sub family but, motif8 and motif 10 was specific to YSL clade. However, motif 1, motif 2, motif 4, motif 6, and motif 7 were common in both sub-families (Fig. 3d). To certain how tomato OPT genes responded to a stress, a 1kb 5`UTR sequences was used to predict presence of various stress-related regulatory sequences in Plant CARE database. We found hormone-responsive regulatory elements, ABRE, ERE, TGA-element, TATC-box, TCA-element, and CGTCA/TGACG-motif, associated with

abscisic acid, gibberellin, auxin, salicylic acid, and methyl jasmonate responses were identified in SlOPTs. Moreover, stress-responsive regulatory elements associated with defense/ stress and low-temperature responses (TC-rich repeats and LTR) were also identified in the promoter sequences (Fig. 4).

Tissue/organ specific expression profiling of SlOPT genes: To investigate biological role of SlOPT in tomato plant growth and development, we have performed a tissue/organ specific expression analysis in various plant parts including different stages fruits. The expression profile of the SlOPT genes significantly varies among different plant parts but no expression of SlOPT4, SlOPT7 and SlYSL6 detected. Is was observed that some genes showed more expressions or expressed in specific plant part than other while, others express in all parts of plant with varying expressions. For example, SlYSL4 and SlYSL5 of YSL sub-family and SlOPT1 and SlOPT2 of PT sub-family showed high expression levels in root tissues but SlYSL1, SlOPT3 and SlOPT5 expression was limited to flowers only. Moreover, SlYSL7 and SlYSL8 have more transcript abundance in leaf than in other plant parts (Fig. 5).

It was also noticed that some genes expressed only at specific stage of fruit development but, others showed varying degrees of expressions. For an instance, SlOPT1, SlYSL2, SlYSL5, and SlYSL8 showed high expressions in mature green fruit but, SlYSL4 and SlYSL7 had high transcript levels in ten days breaker fruit. Additionally, SlYSL3 and SlOPT6 exhibited descending order of expression from 3cm/2cm fruit to ten-day breaker fruit while, opposite trends were observed for SlYSL4 only (Fig. 5). These finding suggesting that tomato SlOPT genes may play pivotal role in tomato various plant parts development.

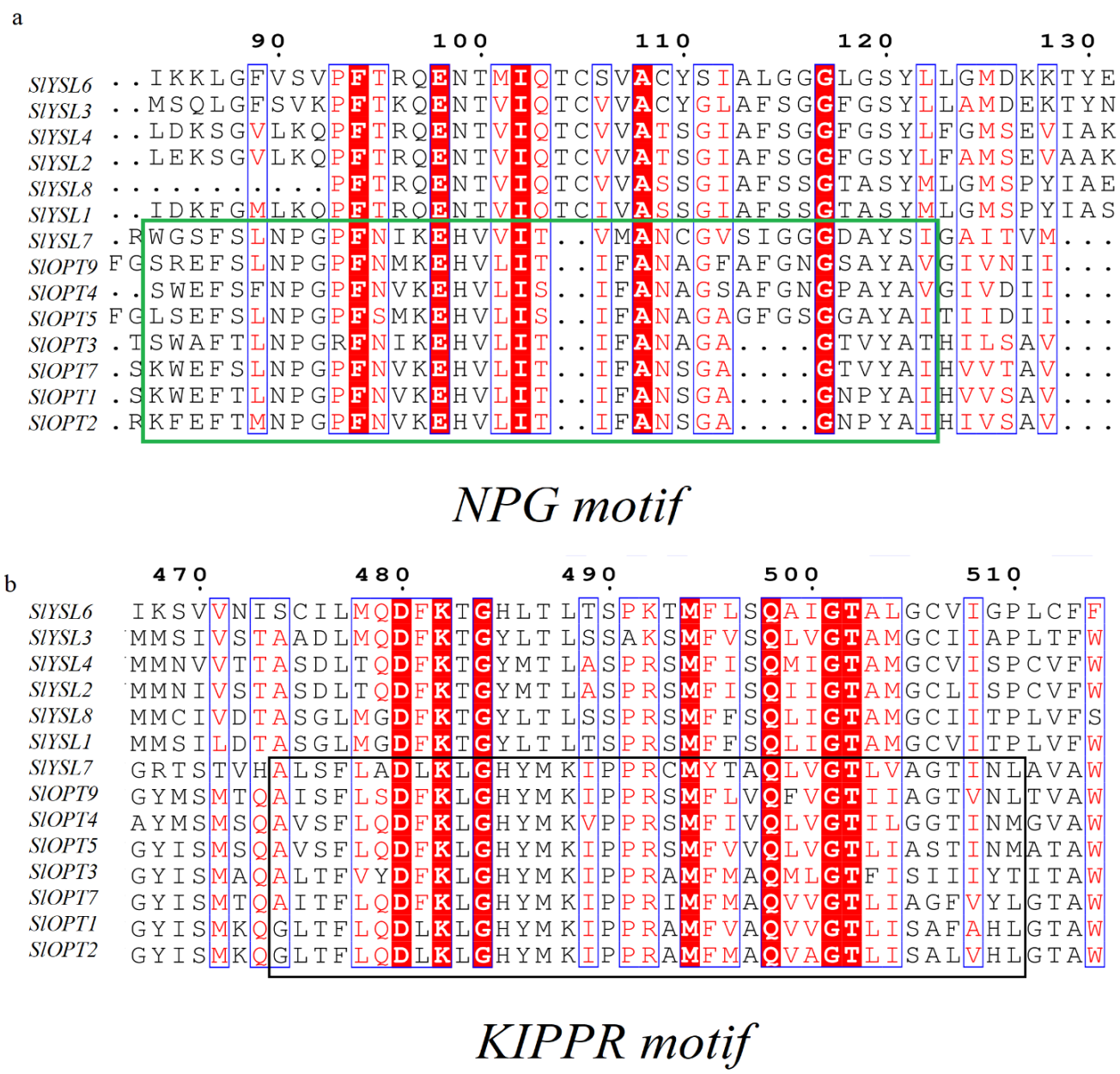

Fig. 1. Multiple sequence alignment of tomato OPT gene family. Tomato OPT (a) the NPF motifs (b) the KIPPR motifs of PT sub-family members.

Table 2. Characteristics of tomato OPT gene family.

	Gene locus ID	Gene name	aa	MW	pI	GRAVY	Chromosome			Exon #	Sub-cellular Localization
							Position	Start	End		
PT sub-family	Solyc02g083740	SlOPT1	733	82.79	7.85	0.428	2	41,608,636	41,613,932	7	plas:11,vacu:2,E.R.:1
	Solyc03g033460	SlOPT2	751	84.42	8.23	0.419	3	9,297,101	9,302,965	6	plas:13,vacu:1
	Solyc03g082700	SlOPT3	739	83.81	8.75	0.433	3	46,163,596	46,167,478	6	plas:14
	Solyc04g076750	SlOPT4	738	82.72	8.72	0.515	4	59,232,077	59,235,653	6	plas:9,E.R.:3,nucl:1,vacu:1
	Solyc04g076760	SlOPT5	746	84.07	8.86	0.463	4	59,239,550	59,242,872	6	plas:14
	Solyc04g076780	SlOPT6	756	85.39	8.58	0.353	4	59,248,010	59,253,947	6	plas:13,vacu:1
	Solyc08g082990	SlOPT7	750	84.45	6.68	0.471	8	62,776,752	62,780,190	7	plas:13,vacu:1
	Solyc11g012700	SlOPT8	756	84.05	9.2	0.425	11	5,469,691	5,473,409	6	plas:11,E.R.:2,vacu:1
YSL sub-family	Solyc02g005340	SlYSL1	653	71.98	8.52	0.438	2	2,271,065	2,273,350	3	plas:9,vacu:3,golg:2
	Solyc02g081570	SlYSL2	694	76.17	8.88	0.447	2	40,045,892	40,050,013	6	plas:9,E.R.:3,cyto:1,vacu:1
	Solyc02g094280	SlYSL3	674	73.53	5.82	0.551	2	49,437,687	49,445,952	7	plas:10,E.R.:2,nucl:1,vacu:1
	Solyc03g031920	SlYSL4	696	76.78	9.44	0.428	3	8,657,524	8,664,052	6	plas:10,E.R.:2,cyto:1,vacu:1
	Solyc03g082620	SlYSL5	658	72.52	9.22	0.5	3	46,082,668	46,086,918	7	plas: 9, golg: 3, vacu: 2
	Solyc05g053110	SlYSL6	580	64.19	7.97	0.54	5	62,373,723	62,375,642	3	plas: 11, E.R.: 2, vacu: 1
	Solyc08g083060	SlYSL7	663	73.26	9.24	0.467	8	62,808,532	62,811,413	7	plas:9,vacu:3,E.R.:1,golg:1
	Solyc09g074960	SlYSL8	606	66.84	8.55	0.425	9	62,259,536	62,261,473	3	plas:9,vacu:2,golg:2,E.R.:1

aa; amino acid, MW; molecular weight, pI; isoelectric point, GRAVY; the grand average of hydropathy respectively

Heavy metals induced expression profiling of OPT genes in tomato: To unveil the biological role of the OPT genes against various metal induced stress, their expression profiles were investigated in tomato seedling (shoot and root) under various heavy metals including calcium, cadmium, iron, lead, magnesium, and positum (Figs. 6, 7 and 8). For calcium treatment, expression of all SlOPTs was induced both in root and shoot but more significant expression in root (Fig. 6a). In shoot, SlOPT2, SlYSL3, SlYSL6, and SlYSL7 was downregulated temporally but, SlYSL5 and SlYSL8 was upregulated. In root SlOPT4 and SlOPT5, and SlOPT6 upregulated while, SlYSL1, SlYSL6, and SlYSL8 downregulated. In comparison, SlOPT2 induced in root but supressed in shoot. Moreover, SlYSL6 was suppressed in both root and shoot. The SlOPT4, SlOPT5, and SlOPT6 were upregulated both in root and shoot but SlYSL3 supressed in shoot (Fig. 6a).

Under cadmium stress, SlOPT2, SlOPT3, SlOPT6, SlOPT7, SlOPT8, SlYSL1, and SlYSL7 upregulated at 12h interval in shoot while SlOPT1, SlOPT2, SlOPT3, SlOPT4, SlOPT7 was downregulated at 24h interval after treatment. In root, SlOPT4, SlOPT5, SlOPT6, SlOPT7, and SlYSL3 has high expressions at all intervals but SlYSL8 and SlOPT3 was supressed at 24h after treatment. By comparing, SlOPT4, SlOPT7, SlOPT7, SlOPT8, SlYSL1, and SlYSL2 were supressed in shoot at 24h interval but upregulated in root tissues. Moreover, for SlYSL7 and SlYSL8 opposite trends were observed both in root and shoot tissues after treatment (Fig. 6b).

Number of genes were induced against iron stress both in root and shoot tissues. For example, SlOPT3, SlOPT4, SlOPT5, SlOPT6, SlOPT7, SlYSL6, SlYSL7, and SlYSL8 was upregulated both in root and shoot. However, SlOPT8 was supressed in shoot but upregulated in root. Similar trends observed for SlYSL2 but opposite for SlYSL3 and SlYSL1 (Fig. 7a). For lead induced stress treatment, SlOPT3, SlOPT4, SlOPT5, SlOPT6, SlOPT7, SlOPT8, SlYSL1, SlYSl3, and SlYSL7 were induced at 6h after treatment in shoot while, at 24h interval in root tissues. SlYSL1, SlYSL3, SlYSL5, SlYSL6, SlYSL7, and SlYSL8 suppressed at 24h interval in shoot but elevated levels were found in root tissues at same interval of time. Moreover, SlOPT5 and SlOPT6 induced in shoot at 24h but supressed in root tissues. For SlOPT1 opposite trends were detected in both root and shoot (Fig. 7b).

For magnesium induced expression, SlOPT1, SlOPT2, SlOPT3, SlOPT4, and SlOPT5 was upregulated in shoot at 6h after treatment but at 24h in root tissues, SlOPT6, SlOPT7, SlOPT8, SlYSL1, SlYSL2, SlYSL3, and SlYSL4 was upregulated in shoot at 24h. Additionally, SlYSL5, SlYSL6, SlYSL7, and SlYSL8 were induced in root tissues across all intervals. For SlYSL8 opposite trends observed both in root and shoot tissues (Fig. 8a). A very diverse expression pattern was observed against potassium induced expression of SlOPT genes. All genes in PT sub-family were induced in shoot at 24h while, YSL sub-family induced in root tissues at same interval to time. Moreover, in root SlOPT5, SlOPT6, SlOPT7, and SlOPT8 were upregulated at 6h but downregulated in shoot at same internal after treatment (Fig. 8b).

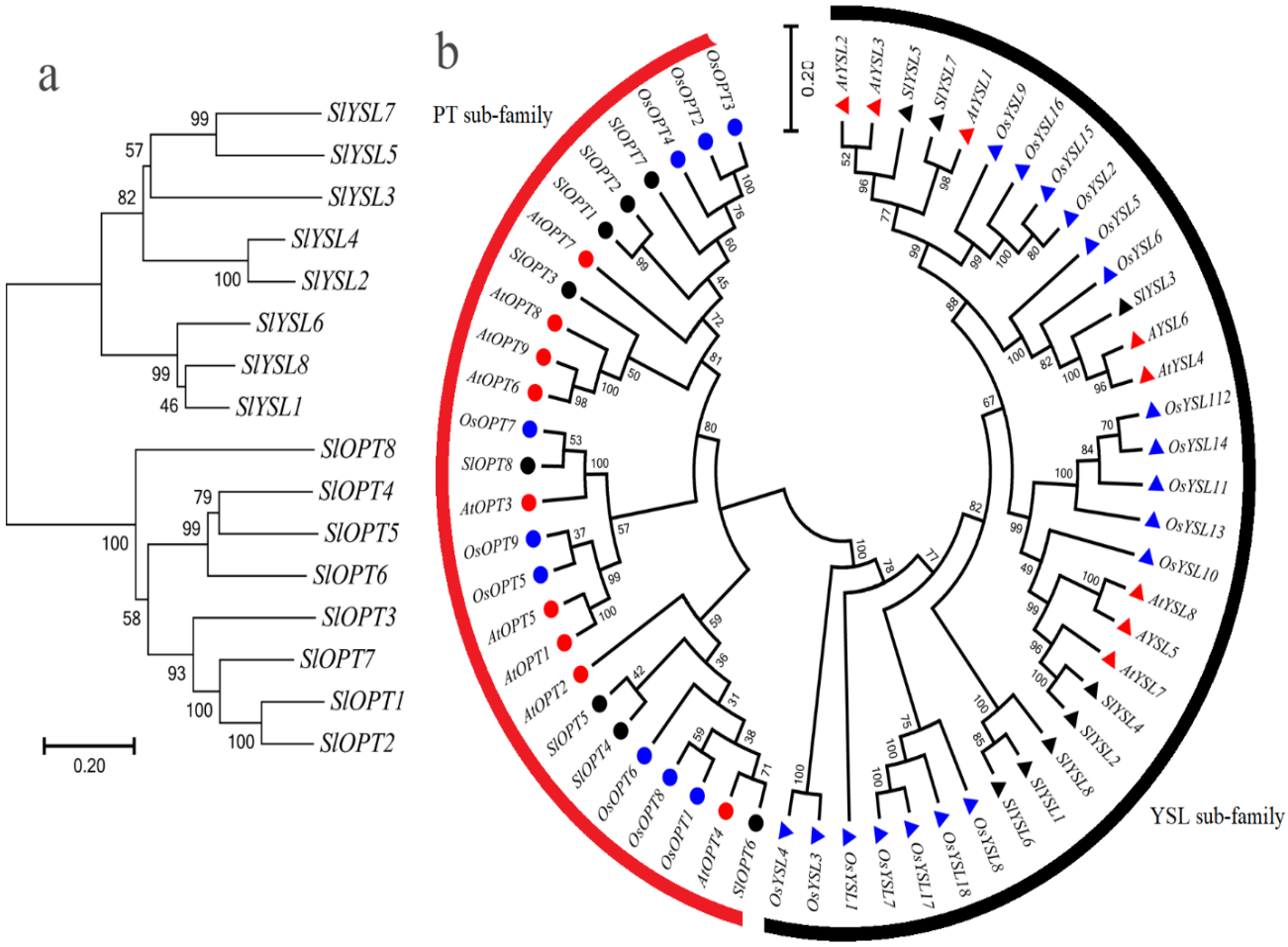

Fig. 2. Phylogeny of OPT gene family. An unrooted neighbour joining phylogenetic tree of (a) tomato OPT family (b) Tomato, Arabidopsis (Koh *et al*., 2002b) and rice genome (Vasconcelos *et al*., 2008) was constructed using MEGAX. SlOPT with blue circle, OsOPT with black circle, AtOPT with red circle, tomato SlYSL with black triangle, OsYSL with blue and AtYSL with red triangle. Red arc represents PT sub-family and black arc corresponding to YSL sub-family of OPT gene family.

Discussion

The OPT transporter proteins play an important role in active transport of ions and other micronutrients from soil to various vegetative or non-vegetative plant parts (Curie *et al.*, 2009) help to maintain ion homeostasis during plant growth and development. in this study, members of OPT gene family was first identified in tomato based on genome wide identification, with their physiochemical features, tissues specific expression and heavy metal induced expression patterns.

Tomato is an ideal vegetable fruit due to its good quality, yield and high nutritive (Seymour *et al.*, 2013) value. OPT genes have been identified and characterized in various plants species including rice (Vasconcelos *et al.*, 2008), *Arabidopsis* (Koh *et al.*, 2002), turnip (Pu *et al.*, 2018), wheat (Kumar *et al.*, 2018), grapes and poplus (Cao *et al.*, 2011). We have identified 16 OPT genes sequences in tomato (Table 1) whole genome based on *Arabidopsis* sequences (Koh *et al.*, 2002). The rice and turnip encode highly hydrophobic polypeptides (GRAVY 0.35-0.525. The tomato OPT genes also encode highly hydrophobic proteins with molecular weight varies from 580 to 756 kDa and average GRAVY ranges from 0.353 to 0.551, sharing high similarly with rice and turnip. This showed that these proteins are highly conserved and positively charged (Table 1).

The OPT genes are classified into two clades based on evolutionary analyses including, PT sub-family and YSL sub-family (Vasconcelos *et al.*, 2008). Tomato OPT genes family division is also consistent with previous analyses. We have found that tomato OPT genes in YSL sub-family contained 3-7 exons and PT sub-family have 4-6 exons (Fig. 3c), like rice, Arabidopsis, turnip, wheat, grapes and poplus. This diverse gene configuration in different phylogenetic clades may exhibit gene family originated from multiple ancestry. The OPT proteins have been identified and characterized in heavy metal detoxification (Cagnac *et al.*, 2004), germination (Müntz, 1998), embryo development (Stacey *et al.*, 2008), and nitrogen translocation (Williams & Miller, 2001). In tomato, the tissue specific expression analysis revealed that SlYSL3, SlYSL4, SlYSL5, SlYSL6, SlYSL7, and SlYSL8 expressed ubiquitously in all plant parts while, others expressed in specific tissues such as root and flowers (Fig. 5). These finding suggested that these proteins may involve in plant growth and development as well.

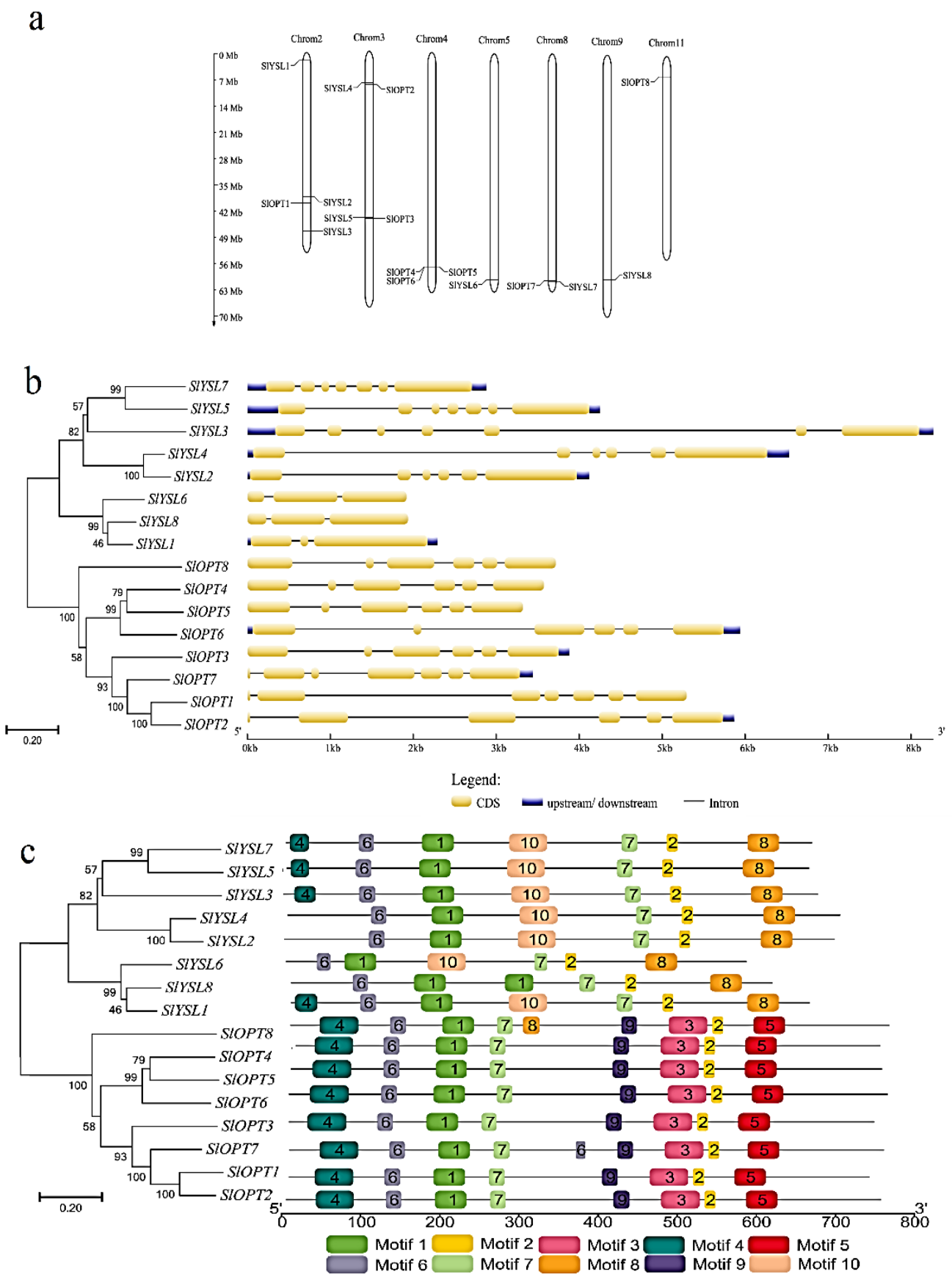

Fig. 3. Physical location, exon/intron distribution, and conserved motif predicted in tomato OPT gene family members. (a)Chromosome location map of tomato OPT genes. The scale of chromosomes is in megabases (MB). (b) Tomato OPT gene intron and exon configuration. The identical exon formation of SlOPT genes is connected with slashes. The scale at the bottom is corresponding to gene size in kb. (c) Prediction of conserved motifs in tomato OPT gene family. MEME identified motifs and length of each motif indicated proportionally. Each color of box is corresponding to a motif.

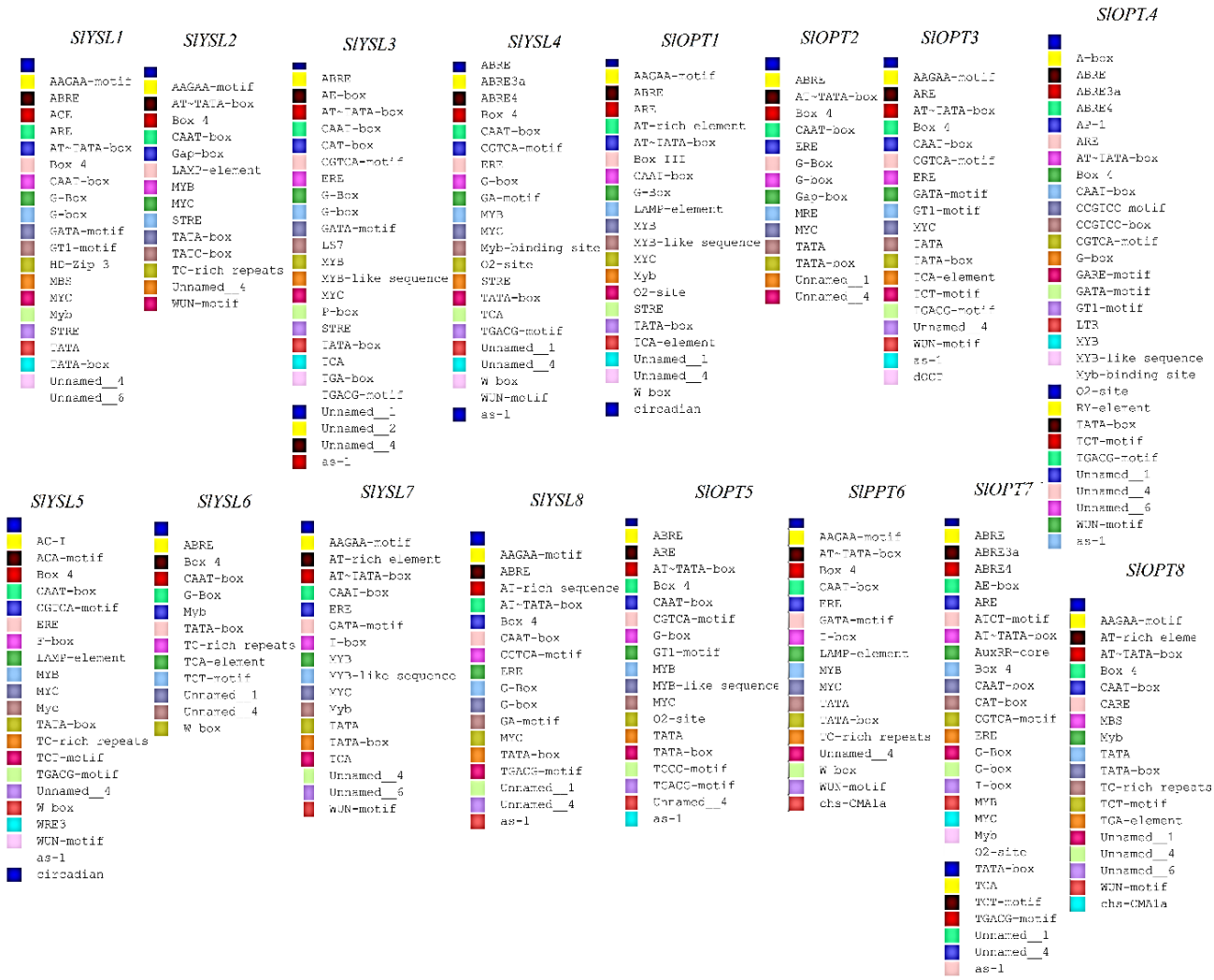

Fig. 4. The putative cis-regulatory elements predicted in tomato OPT gene family members using PlantCARE database.

To date, no distinct heavy metal induced expression profile analysis have been conducted in tomato. In this study, we uncovered the heavy metal tolerance mechanism, laying foundation for exploring the physiological and biochemical role of OPT genes in tomato. Heavy metals like cadmium, calcium, iron and so on pollute the agricultural land therefore, affecting plant growth and development that subsequently resulted in lower yield and productivity the OPT proteins play important role in metal ion absorption and entry into the food chain. We have investigated, the expression profile of tomato OPT proteins under heavy metals stress (Figs. 6, 7 and 8). The expression analysis revealed that most of SlOPT genes expression levels were changed in response to these metals. For example, under calcium stress, SlOPT4, SlOPT5, and SlOPT6 of PT sub-family upregulated in both root and shoot but SlYSL5, SlYSL8, SlYS4 in YSL sub-family was downregulated (Fig. 6a). In case of cadmium, OPT proteins expressed temporally. For an instance, SlOPT2, SlOPT6, SlOPT7, SlYSL1, and SlYSL7 induced at 12h interval only but SlOPT1, SlOPT2, and SlOPT3 was downregulated at 24h after treatment. Similarly, some gens show opposite trends like SlYSL7 and SlYSL8 (Fig. 6b).

SlOPT3, SlOPT6, SlOPT7, SlYSL8, and SlYSL7 was upregulated in both root and shoot under iron stress but, SlOPT4, SlOPT5, SlOPT8, and SlYSL1 induced in shoot at 6h after treatment. Moreover, SlOPT5 and SlOPT6 was supressed in root against lead stress but SlOPT8 was upregulated in root under iron treatment (Fig. 7a). For potassium, a very unique expression pattern was observed. All PT clade members were induced in shoot and YSL clade members were induced in root at 24h after treatment (Fig. 8b). SlOPT1, SlOPT2, SlOPT3, SlOPT3, and SlOPT5 in root and YSL1, SlYSL2, SlYSL3, and SlYSL in shoot were upregulated at 24 intervals under magnesium (Fig. 8a). These considerable differences in expression among tomato OPT genes suggest that these genes involved in various physiological and biochemical processes to help plant adopt under complicated circumstances.

Taken together, this study provides new information about the OPT gene family in tomato genome including various gene characteristic properties, conserved motif prediction, cis-regulatory sequences prediction, chromosome location and gene intron/exon configuration. Moreover, the expression profiling in various plant parts and heavy metal induced expression analysis helped in understanding the biological role of these gene in tomato plant growth, development, and their physiological role under stresses conditions.

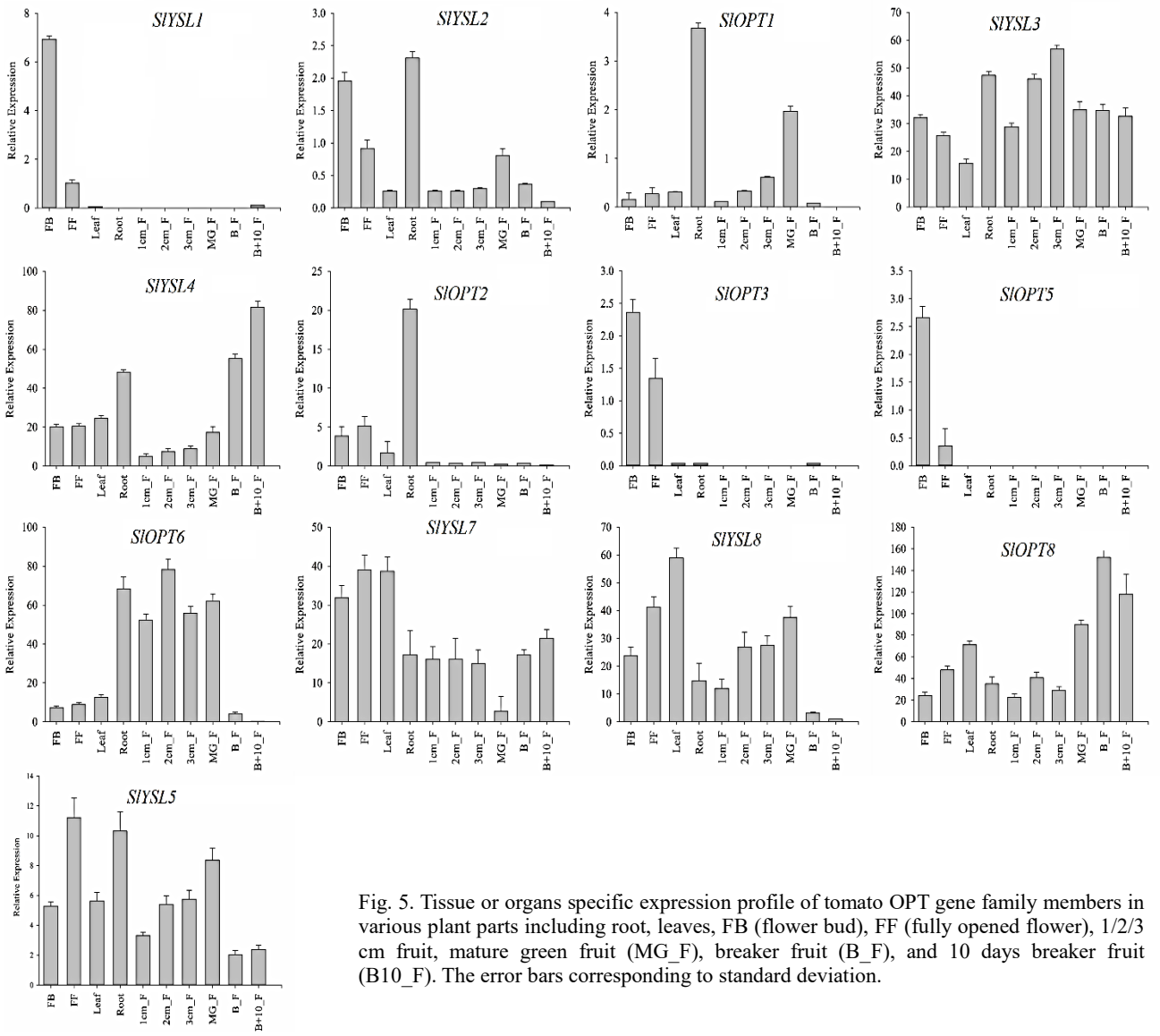

Fig. 5. Tissue or organs specific expression profile of tomato OPT gene family members in various plant parts including root, leaves, FB (flower bud), FF (fully opened flower), 1/2/3 cm fruit, mature green fruit (MG_F), breaker fruit (B_F), and 10 days breaker fruit (B10_F). The error bars corresponding to standard deviation.

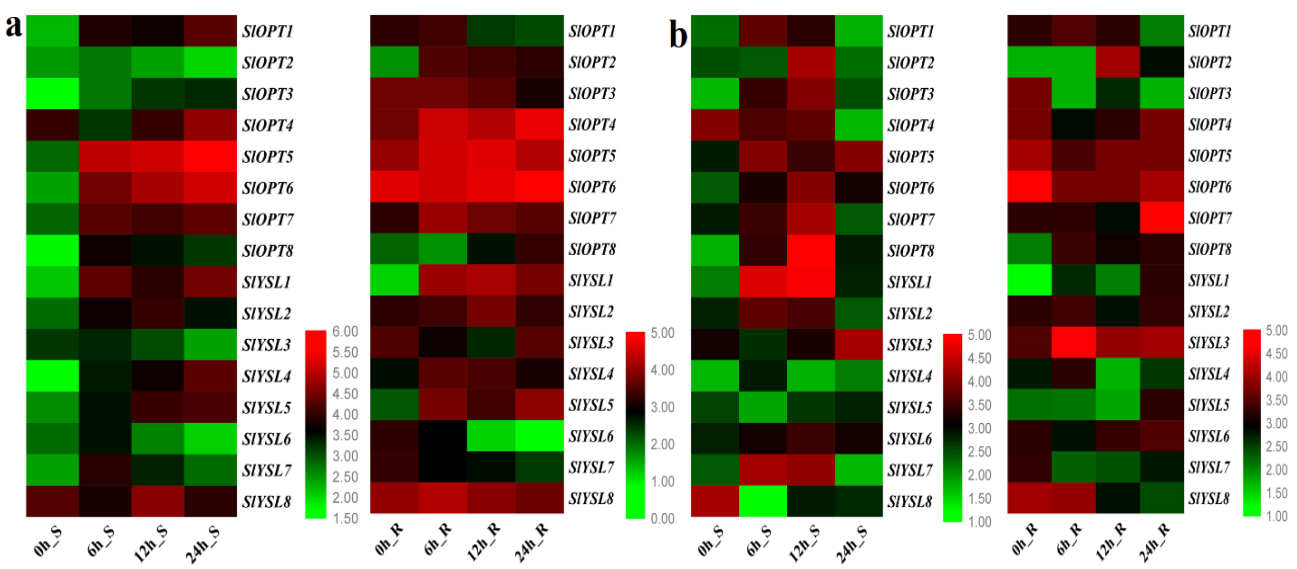

Fig. 6. Expression profile of tomato OPT family under (a) calcium (b) cadmium in shoot and root of tomato seedlings. _S; shoot, _R; root. Green, black, and red color represent lowest, moderate, and highest expressions, respectively.

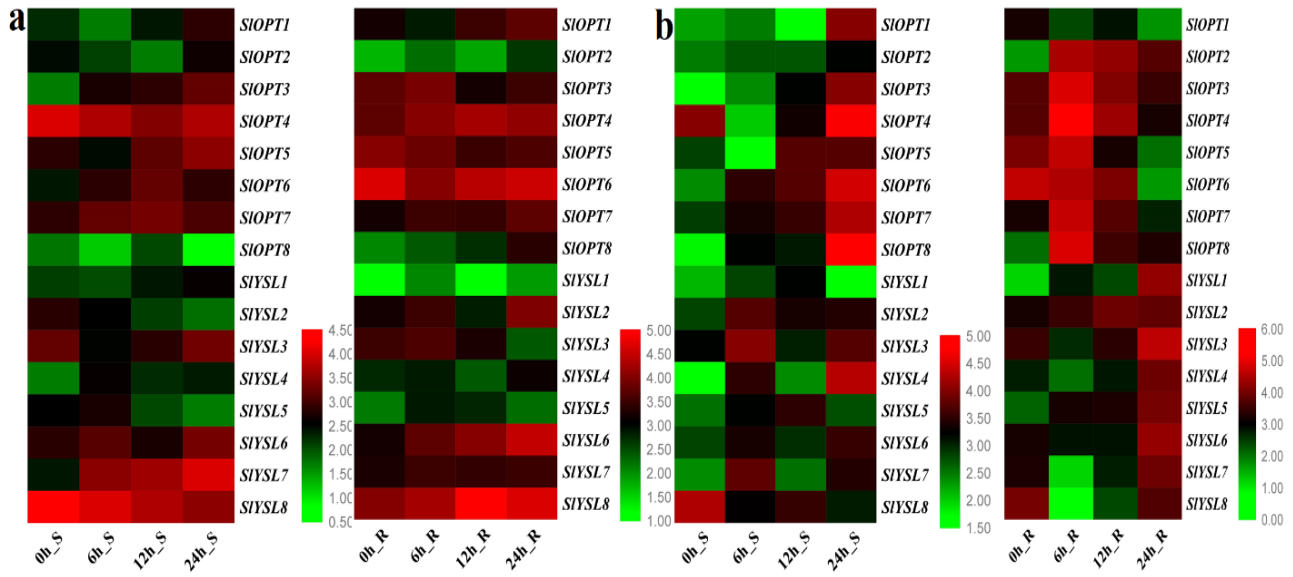

Fig. 7. Expression profile of tomato OPT family under (a) iron (b) lead in shoot and root of tomato seedlings. _S; shoot, _R; root. Green, black, and red color represent lowest, moderate, and highest expressions, respectively.

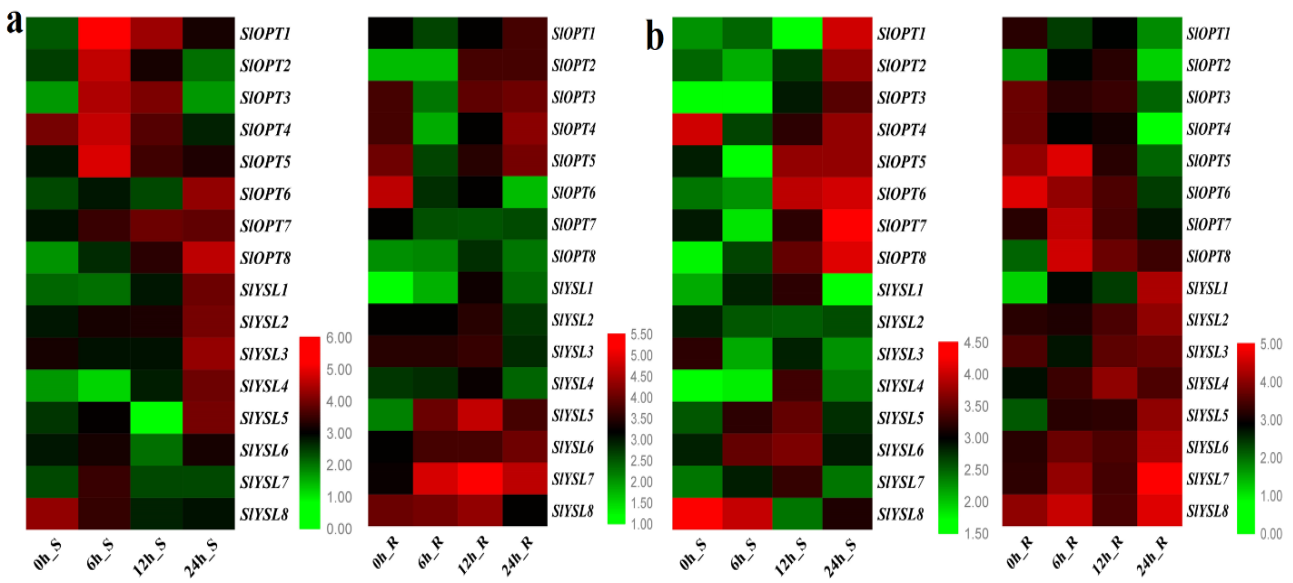

Fig. 8. Expression profile of tomato OPT family under (a) magnesium (b) potassium in shoot and root of tomato seedlings. _S; shoot, _R; root. Green, black, and red color represent lowest, moderate, and highest expressions, respectively.

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