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TRANSCRIPT PROFILING OF ZIP GENES TO UNZIP THEIR ROLE IN ZINC ASSIMILATION IN *Solanum lycopersicum* (L.)

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Zinc (Zn) is needed for plants growth and human's balanced diet. The non-availability or less mobilization of Zn affects crop yield and nutritional quality and when such produce is consumed, it causes malnutrition in the consumers. Biofortification of staple cereals and vegetables like tomato (*Solanum lycopersicum* L.) is one of the strategies to fight such type of hidden hunger, particularly to crumble Zn deficiency. This needs the understanding of the molecular mechanism of Zn assimilation in plants. ZIP genes have not been annotated and characterised earlier. Therefore, the genes of ZIP family were identified from genome database by homology search. After carrying out the phylogenetic analysis, the 10 diverse genes of tomato ZIP family were selected for the transcript profiling in two selected genotypes; one with the highest Zn assimilation (LA-2662) and the other with the lowest one (NTH-242) was done. The RT-PCR results showed that in genotype LA-2662, genes SLZIPL, SLZIP3 SLZIP5L and SLZIP5 were upregulated in roots and leaf tissues, while their transcript level was the lowest in genotype NTH-242. In addition, the genes; SLZIPL, SLZIP3, LeIRT1 and LeIRT2 were upregulated under Zn deficient environment and downregulated in high Zn environment. The better performance of LA-2662 for Zn assimilation might be linked to the upregulation of SLZIPL and SLZIP3 genes. It showed that the attenuation of these candidate genes might improve the capability of tomato for the development of Zn fortified tomato genotypes. It further showed that tomato can be bred for biofortification of various nutritional elements to fight hidden hunger.
Key words: Biofortification, tomato, zinc accumulation, SLZIPs gene, gene regulation.

INTRODUCTION

Food security is becoming a point of concern, particularly in Asia due to exponential population growth. There has been a significant increase in food production in the past half century to feed the ever increasing population, still there is one out of seven people who doesn't have access to sufficient calories and protein and suffer from micronutrient deficiency. In fact, for the first time in human history, the undernourished people number increased to 1 billion (Garrity *et al*., 2010; Godfray *et al*., 2010). Balanced and healthy diet is an important factor in preventing chronic diseases such as cancer, neuro-degenerative and cardio-vascular diseases (Dorais *et al*., 2008). The use of tomato (*Solanum lycopersicum*) in human diet is increasing and considered as a healthy diet because of high content of lycopene and other health promoting natural compounds.

Plants need various essential metal ions (Zn^{2+}, Fe^{2+}, $Ca^{2+,}$ Mg^{2+}, Sr^{+}, $Mn^{2+}$and Ba^{2+}) for their optimal growth and survival and these metal ions should be transported from soil to plant roots and then distributed throughout the plant, in both cellular and organelle membranes. These metal elements are present in trace amount in soil solution so, plant needs high affinity transport system to accumulate these metal ions in tissues. A number of genes have been identified which are involved in transportation of these metals. Some genes were identified by functional complementation of yeast mutants and others were found on the basis of sequence similarity by using a variety of approaches including database mining, degenerate polymerase chain reaction and heterologous hybridization techniques (Axelsen and Palmgren, 1998). Most of these genes belongs to the already studied transporter families like P-type ATPase and NRAMP proteins.

Recent studies on Arabidopsis related to metal transportation have identified a new family of metal transport i.e. ZIP family, which help in the transport of basically four essential micronutrients (Cu, Fe, Zn and Mn) in plant roots (Matthew *et al*., 2013). ZIP family takes its name from identification of first member 'ZRT, IRT like protein' whereas IRT (iron regulated transporter) has been identified as Arabidopsis cation transporter, expressed in the root cells of iron deficient plants (Eide *et al*., 1996) and ZRT (zinc regulated transporter) protein. The ZIP family proteins are of two types; ZRT1 and ZRT2, which are expressed under high and low zinc affinity in yeast respectively (Zhao and Eide, 1996).

ZIP family has 25 genes, which have been categorized into two subfamilies based on amino acid sequence similarities. Fifteen genes fall into subfamily I, which are present in plants

(11 studied in Arabidopsis, one in pea, two in tomato and one in rice). Subfamily II includes eight genes in nematodes, two in humans and one in Drosophila. Two genes in yeast ZRT1 and ZRT2 have also been added in this family. Structural analysis of ZIP protein showed that this protein has eight transmembrane potential domains and their amino and carboxy terminal ends are present on the outer surface of the plasma membrane.

ZIP protein have a variable region ranging between 309-476 amino acids in length. This length is between transmembrane III and IV and represents the metal binding domain, enriched with histidine residues. The most conserved portion of ZIP protein was found in transmembrane IV with highly conserved histidine residue predicted to form an amphipathic helix. This histidine region along with adjacent polar residue made a part of intramembranous heavy metal binding site that is crucial components of metal transport system (Eng *et al*., 1998)

Zn is required in adequate amount to plants for their better growth and survival and also share adequate place in food chain. The effects of Zinc deficiency on crop yield has become a worldwide concern in terms of food availability and malnutrition (Abelson, 1999). Deficiency of Zn element in plants showing the symptom of interveinal necrosis, deformed and chlorotic leaves, reduced biomass and also cause reduction in yield. The Zn concentration required for healthy growth of plants ranges from 15 to 20 mg/kg dry weight in leaf (Marschner, 1995). On the other side, high level of Zn concentration in growth medium can cause toxicity in plants. At cellular level, high level of Zn causes reduction in the accumulation of ATP, creates oxidative stress, disintegration of cell organelle and enhances number of vacuoles in the cell (Sresty and Madhava Rao, 1999; Xu *et al*., 2013).

The important steps of Zn transport in plants is controlled by many genes, which have been identified and characterized at molecular level by many researchers. One of the major Zn transporter families studied was ZIP (ZRT-IRT like protein) (Sinclair and Kramer, 2012) and it has 15 members in *Arabidopsis thaliana*. The expression studies were also done in two species of Arabidopsis i.e. *A. thaliana* and *A. halleri*, which showed that mutant of *A. thaliana* to IRT gene accumulated less zinc as compared to its wild type, indicating the role of IRT gene in Zn transport (Henriques *et al*., 2002). Studies on expression level in root tissues of *A. thaliana*, showed that ZIP genes like ZIP1, ZIP3 and ZIP4 are expressed under Zn deficient condition while ZIP1, ZIP3, ZIP4, ZIP9 and ZIP10 are overexpressed in *A. halleri* as compared to *A. thaliana*. This result indicated that *A. halleri* is more Zn responsive species (Talke *et al*., 2006).

In this modern era consumers are very discriminating in their eating habits and the demand for healthier food is increasing day by day due to malnutrition problems especially in children. Both plant breeders and biotechnologists are beginning to realize that the development of genotypes with improved nutrition contents is as important for consumer as producing a high yielding variety for the farmers. But this needs better understanding of physiological and molecular mechanism of Zn assimilation in tomato. While the Zinc sensing and its transmission in vegetable plant like tomato is poorly understood although the tomato genome has been thoroughly sequenced and assembled. But the understanding of molecular mechanism of different cation transporter genes is still limited. Therefore, the aim here was to study molecular mechanism of important cation (Zn^{2+}) transporter genes family in tomato. The Zn transporter genes in tomato were identified along with their location on chromosome and their expression pattern in different tissues at different stages under different Zn environments.

MATERIALS AND METHODS

***Plant material*:** Two genotypes of tomato, NTH-242 and LA-2662 were planted in a greenhouse of Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad in 2016-17, where proper light, air and water system was managed. First the seed of these genotypes were sown in sand filled pots. For expression analysis of Zn transporter genes, one month old seedlings were transferred to hydroponic medium and three Zn treatments in the form of zinc sulphate were applied at the time of transplantation in hydroponic medium (T_1=control, T_2= 40PPM and T_3= 100PPM). Hoagland medium was prepared following Jensen and Malter (1995), and it was replaced after every three weeks to maintain the concentration of nutrients in the tub. The pH of hydroponic solution was maintained at 6 to 6.5 adding either H_2SO_4 or NaOH.

***Sample collection for RNA Extraction*:** Samples were taken both from leaf and root tissues of the genotypes, NTH-242 and LA-2662 at two stages of plant. Firstly, leaf and root tissue samples were taken after one week of seedling transplanting in hydroponic media (at early stage), then leaf and root tissue samples were taken at maturity stage. Samples were taken in autoclaved Eppendorf tubes using sterilized scissor and distilled water was used to remove the dirt from sample and then wrapped in tissue paper to absorb moisture.

***RNA extraction and first strand cDNA synthesis*:** Total RNA was extracted using thermo scientific GeneJet RNA purification Kit (USA) as shown in Figure 1 and 2. Extracted mRNA from both leaves and roots was converted to cDNA (complementary DNA) with the use of Revert Aid First Strand cDNA synthesis Kit (Fermentas, USA) by using oligo-dt primers.

***Quantitative real time PCR of SLZIPs genes*:** Synthesized cDNAs were used as template for relative quantification of the transcripts of zinc transporter genes. Normalized expression of zinc transporters genes was measured by real

Table 1. Primers used for the amplification of *ZIP* genes using real time PCR

Gene Name	Primer Name	Sequence 5'—3'	Amplicon Product Size (bp)
Solanum lycopersicum Ubiquitin1(internal control)	SLUBI-F	5' CCAAGATCCAGGACAAGGAA 3'	183
	SLUBI-R	5' AAGCCTCTGAACCTTTCCAG 3'	
Solanum lycopersicum zinc transporter-like	SLZIPL F	5'GATCCCAAATCGCCCTTTAC3'	236
	SLZIPL R	5'GAGGCTTGTCAATCTTGACC3'	
Solanum lycopersicum zinc transporter	SLZIP2 F	5'GAAGGCATTGCTGTTGGAGT3'	179
	SLZIP2 R	5'GGGCTTGAAATTGCAAAGGC3'	
Solanum lycopersicum zinc transporter 3-like	SLZIP 3 F	5'GGTGGATGTATTTCCCAGGCAA3'	209
	SLZIP 3R	5'AGTAGATCCACGAGTGCCATGT3'	
Solanum lycopersicum zinc transporter 4, chloroplastic	SLZIP 4F	5'TGGTCATTCCCACTCCCATAGCTT3'	180
	SLZIP 4R	5'TGGAATGACAACGCTACGAGCAAG3'	
Solanum lycopersicum ZIP5	SLZIP 5F	5'AGGGATGTCGCAGAATCAGT3'	226
	SLZIP 5R	5'TGCATTTGGGCTTGTATCAT3'	
Solanum lycopersicum zinc transporter 5-like	SLZIP5LF	5'GTGGATGCATAGCTCAGGCAAA3'	179
	SLZIP5LR	5'AATGCCAGCTGATGCCGAAT3'	
Solanum lycopersicum zinc transporter 5-like 2	SLZIP5L2F	5'TTGAAGGCATGGGACTTGGT3'	199
	SLZIP5L2R	5'AAGATGCCAGCTGAAGCTGA3'	
Solanum lycopersicum iron-regulated transporter 1	LeIRT1F	5' GTTTGAAGGAATGGGCCTTG3'	125
	LeIRT1R	5' ACAATGCTATCCCAAGTGCT3'	
Solanum lycopersicum iron-regulated transporter 2	LeIRT2F	5' TGCTGCACTTTGCTTTCATC3'	121
	LeIRT2R	5'TGGAGTTGTTACTGCGAAGA3'	

time analysis using 2X Syber Green Super Mix (Fermentas, USA).

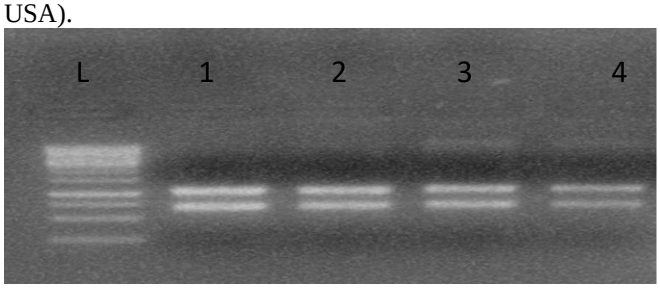

Figure 1. Total RNA Extracted from leaves and roots of tomato genotypes under 40 PPM Zn Treatments at maturity stage. L= 1Kb DNA Ladder. The quality of RNA was determined by running on 1% agarose gel. 1= NTH-242 (leaf sample), 2= LA-2662 (leaf sample), 3= NTH-242 (root sample), 4= LA-2662(root sample).

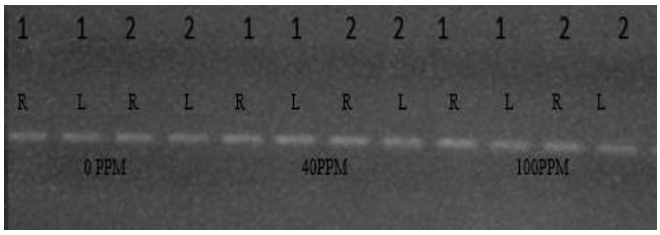

Figure 2. RT-PCR for SLUBI gene in tomato genotypes at various Zn treatments in root and leaf tissues. 1= NTH-242, 2= LA-2662 genotype. R= root sample, L= leaf sample.

Each PCR plate containing 96 wells had three replications for each level of zinc treatment of each plant part at each stage. Real time PCR was performed in iQ5 cycler CFX96 (BioRad, USA) using designed primers (Table 1) and SLUBI was used as internal control gene (Fig. 2). The specificity of the amplicon was confirmed by melt curve analysis (55 ^{o}C to 95 ^{o}C) and then electrophoresed on agarose gel.

RESULTS

***Identification of ZIP genes in tomato and comparison with ZIP genes homologs present in other species*:** A total of 14 tomato ZIP genes were identified using reported Arabidopsis ZIP gene as query in TBLASTN. These 14 genes have complete coding sequence. They were designated as SLZIP 1-8 and SLIRT1-2 according to the amino acid sequence similarity with Arabidopsis genes (Table 2). The predicted ZIP genes in tomato have amino acids between 276-598 similar to conserved domains of ZIP protein (Table 2). In addition, these genes also contained a variable region between transmembrane domains where potential metal binding takes place. The phylogenetic analysis was done by using the reported ZIP genes of different species *(Arabidopsis Thaliana, Oryza sativa, Zea mays, Glycine max, Homo sapiens and Saccharomyces cerevisiae). The analysis* showed that SLZIP1 was closely related to AtZIP2 and OsZIP1, while SLZIPL was related to AtZIP11, OsZIP2 and GmZIP11. In addition, *Solanum lycopersicon* ITR1 and IRT2 form gene cluster with AtIRT1, AtIRT 2 and with AtZIP12 (Fig. 3).

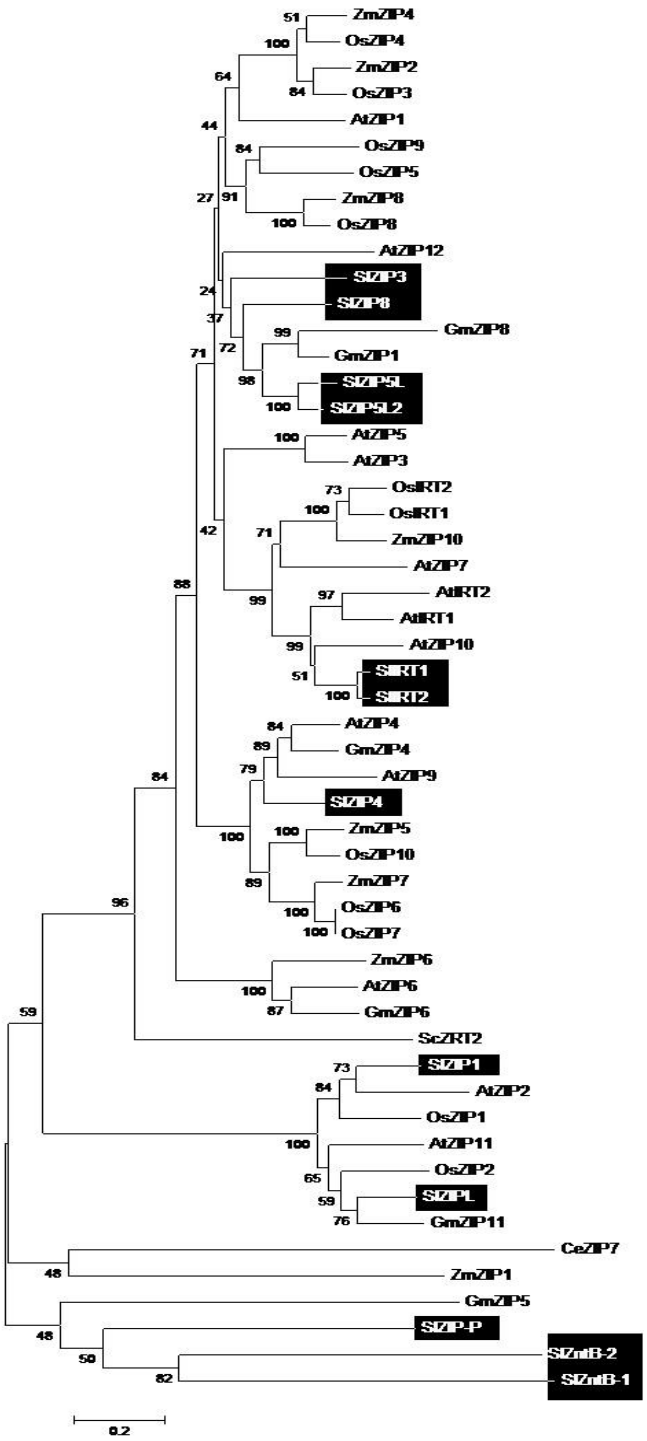

Figure 3. Phylogenetic analysis of ZIP gene family members from *Arabidopsis thaliana*, maize, rice, soybean, yeast, nematode and human with *ZIP* genes of *Solanum lycopersicon* species. The un rooted phylogenetic tree was constructed with the deduced protein sequences of ZIP proteins using neighbour-joining method in MEGA-6 software.

This phylogenetic tree showed that genes of ZIP family of different species have conserved amino acid sequences among them, which represent the same functional characteristics of Zn and Fe transporters.

***Transcription pattern of SLZIPs genes in leaf tissue of tomato plant at vegetative stage*:** The expression pattern of SLZIPs gene was studied in different tissues of tomato at different stages under various zinc treatments. Quantitative PCR showed that when different levels of zinc were applied, differential expression of ZIP genes were observed in both tissues i.e. leaf and root. Under controlled conditions, at 0 PPM level of Zn, the accumulation of SLZIP3, SLZIP5 and SLZIP5L gene transcripts was higher in leaves as compared to other targeted genes at vegetative stage in NTH-242 genotype. At 40 PPM level of Zn, the SLZIP5L transcripts were higher and no transcription of SLZIP2 gene was found at vegetative stage while only SLZIP3 was found to be up-regulated at higher level of zinc concentration i.e. at 100 PPM in NTH-242 genotype (Fig. 4).

In LA-2662 genotype, under controlled conditions the transcription of only SLZIP5 gene was observed while SLZIP5L2 gene transcription was down regulated as compared to others. While at 40 PPM level of zinc, transcripts of two genes SLZIPL and SLZIP3 were higher in leaves at vegetative stage and genes SLZIP3 and SLZIP5 showed their transcription pattern higher than other studied genes at 100 PPM level of zinc in leaves tissues. This showed that SLZIP3 transcript was sensitive to Zn environment in leaf in both genotype and the transcription pattern of SLZIP4, SLZIP5L2, LeIRT1 and LeIRT2 showed no obvious change at different zinc levels (Fig. 5)**.**

***Transcription profiling pattern of SLZIP gene in root tissue of tomato plant at vegetative stage*:** A varying pattern of SLZIP3 gene transcription was observed in root tissue under different Zn levels in NTH-242 genotype. The transcription of SLZIP3 gene was three times higher at 100 PPM zinc concentration as compared to control and 40 PPM. The accumulation of gene transcript SLZIPL was up-regulated under high zinc concentration. Iron regulated gene LeIRT1 also transcribed in root tissue under controlled conditions while its transcription pattern two times higher under Zn deficient environment as compared to 40 PPM and 100 PPM level and LeIRT2 gene transcription pattern was also higher under Zn deficient environment and decreases as the level of zinc increases. In root tissues, the transcription of SLZIP2, 4, 5, 5L, 5L2 genes were low up regulated at 100 PPM and very low transcripts were observed under both control and 40 PPM zinc level (Fig. 6).

In LA-2662 genotypes similar transcription pattern of ZIP genes was observed as in NTH-242 genotype. The accumulation of transcript SLZIP3 gene was up-regulated at 0 PPM level of zinc and two iron regulated genes LeIRT1 and LeIRT2 were up-regulated as the zinc concentration decreases in soil. While the transcription of SLZIPL down regulated in

root tissue as the zinc level increases in the growth medium. The transcription pattern of SLZIP2, SLZIP4, SLZIP5, SLZIP5L, SLZIP5L2 showed same transcription level at all zinc levels (Fig. 7).

***Transcription profiling pattern of SLZIP gene in leaf tissue of tomato plant at flowering stage*:** Results of Quantitative PCR showed that when different levels of zinc were applied the differential transcription of target gene was observed in both tissues i.e. leaf and root. Under controlled conditions at 0 PPM, the accumulation of SLZIPL and SLZIP3 gene transcripts was higher in leaves as compared to other targeted genes at maturity stage in NTH-242 genotype. At 40 PPM and 100 PPM only SLZIPL gene transcription pattern was high as compared to other studied gene and little transcription of other ZIP genes SLZIP2, SLZIP3, SLZIP4, SLZIP5, SLZIP5L, SLZIP5L2 was found at maturity stage. Whereas the transcription profiling of LeIRT1 and LeIRT2 was high under zinc deficient medium and its transcription decreases as the level of zinc decreases in the growing medium (Fig. 8).

Table 2. List of ZIP genes in tomato

Gene name	Gene ID	NCBI accession	CDS Length	Protein Length	Location on Chromosome	Conserved Domains
SLZIP-P	LOC 101251030	XM_010326361.2	1797	598	1	2
SL ZntB-1	**LOC101246165**	XM_004245251.3	1374	457	8	1
SL ZntB-2	**LOC101264769**	XM_004248737.3	1347	448	10	1
SLZIP1	**LOC101255999**	NM_001322833.1	1013	337	6	1
SLZIP6	**LOC100750256**	XM_010322986.2	987	328	5	1
SLZIPL	**LOC100037509**	NM_001247420.1	1053	350	7	1
SLZIP3	**LOC101260003**	XM_004232601.3	1059	352	2	1
SLZIP4	**LOC101259773**	XM_004245052.2	1224	407	8	1
SLZTP-29	**LOC101253965**	XM_004250824.3	831	276	11	1
SLZIP5L	**LOC101257981**	XM_004243601.3	1029	342	7	1
SLZIP8	**LOC101252338**	XM_004231552.3	1044	347	2	1
SLZIP5L2	**LOC101248893**	XM_004243848.3	1029	342	7	1
LeIRT1	**LOC543597**	NM_001247319.1	1053	350	2	1
LeIRT2	**LOC543598**	NM_001247323.1	1059	352	2	1

Note: CDS of each predicted gene sequence was obtained from Genbank by BLAST search.

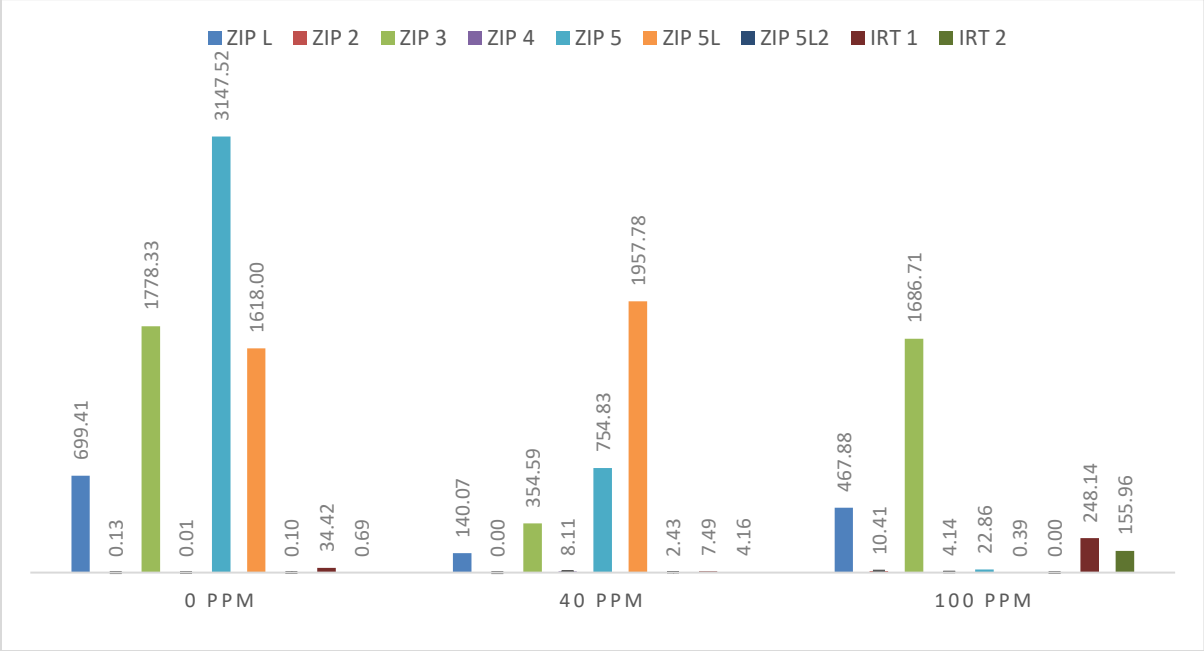

Figure 4. Relative profiling of Different Zinc Transporter genes transcripts in leaf In Genotype NTH-242 at 0, 40, 100 PPM of Zinc levels at vegetative stage. Relative mRNA abundance of each gene was normalized with SLUBI gene. Data from RT-PCR was analysed according to $2^{-\Delta\Delta Ct}$ method. Error bars indicate standard deviation**.**

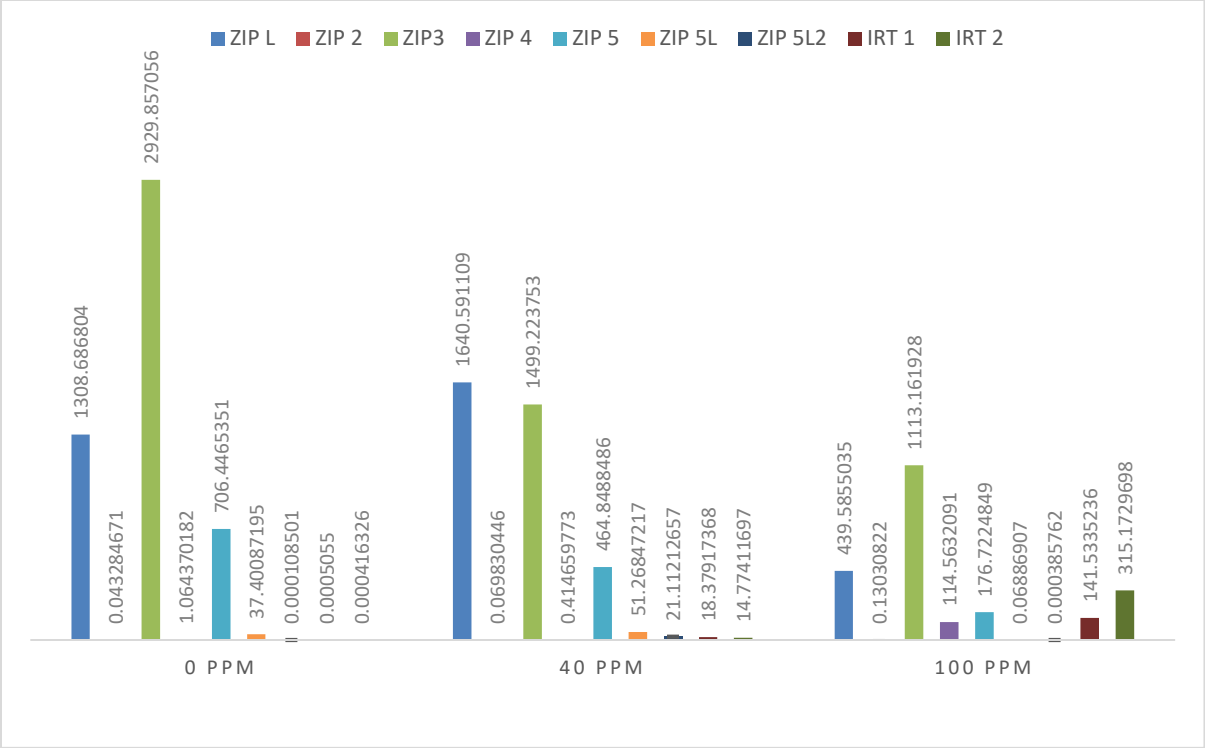

Figure 5. Relative profiling of Different Zinc Transporter genes transcripts in leaf In Genotype LA-2662 at 0, 40, 100 PPM of Zinc levels at vegetative stage. Relative mRNA abundance of each gene was normalized with SLUBI gene. Data from RT-PCR was analysed according to $2^{-\Delta\Delta Ct}$ method. Error bars indicate standard deviation**.**

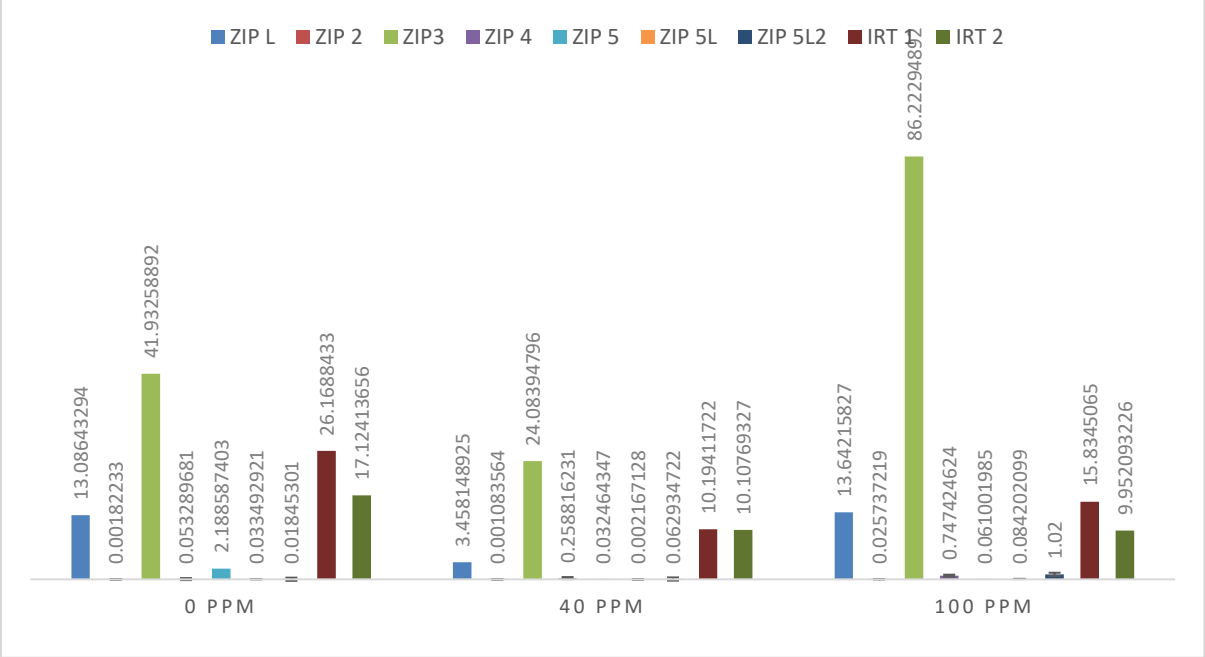

Figure 6. Relative profiling of Different Zinc Transporter genes transcripts in root In Genotype NTH-242 at 0, 40, 100 PPM of Zinc levels at vegetative stage. Relative mRNA abundance of each gene was normalized with SLUBI gene. Data from RT-PCR was analysed according to $2^{-\Delta\Delta Ct}$ method. Error bars indicate standard deviation**.**

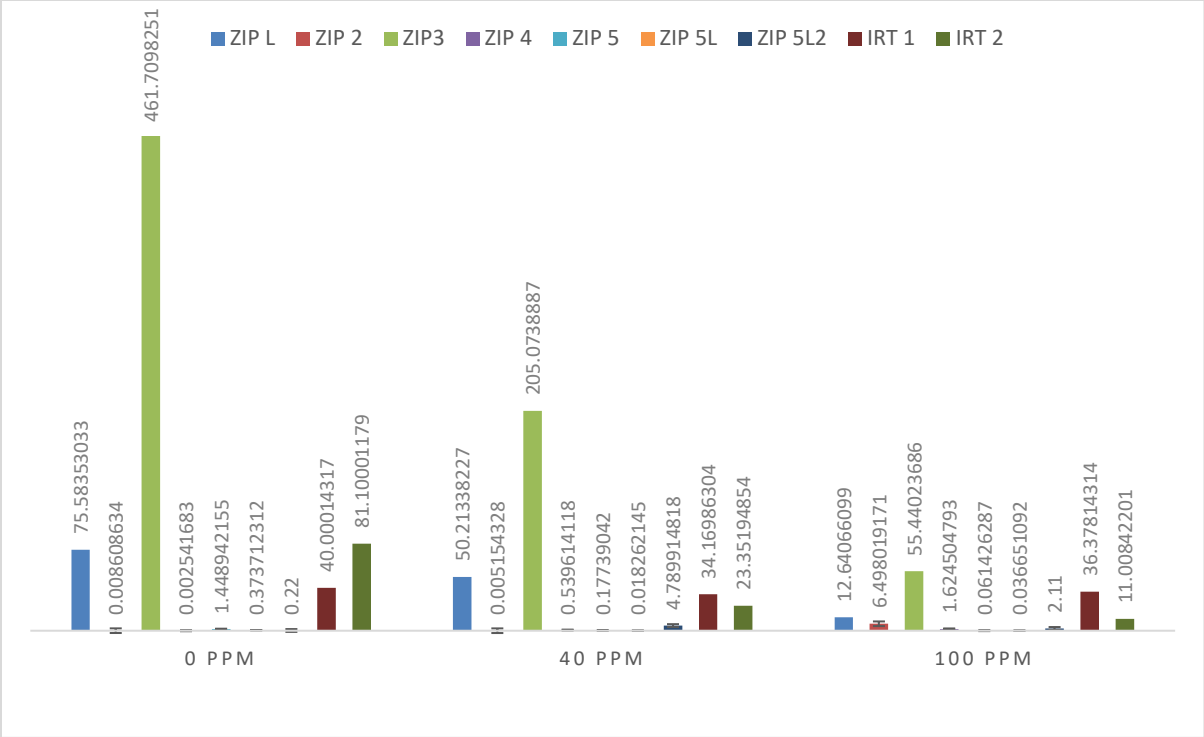

Figure 7. Relative profiling of Different Zinc Transporter genes transcripts in root In Genotype LA-2662 at 0, 40, 100 PPM of Zinc levels at vegetative stage. Relative mRNA abundance of each gene was normalized with SLUBI gene. Data from RT-PCR was analysed according to $2^{-\Delta\Delta Ct}$ method. Error bars indicate standard deviation**.**

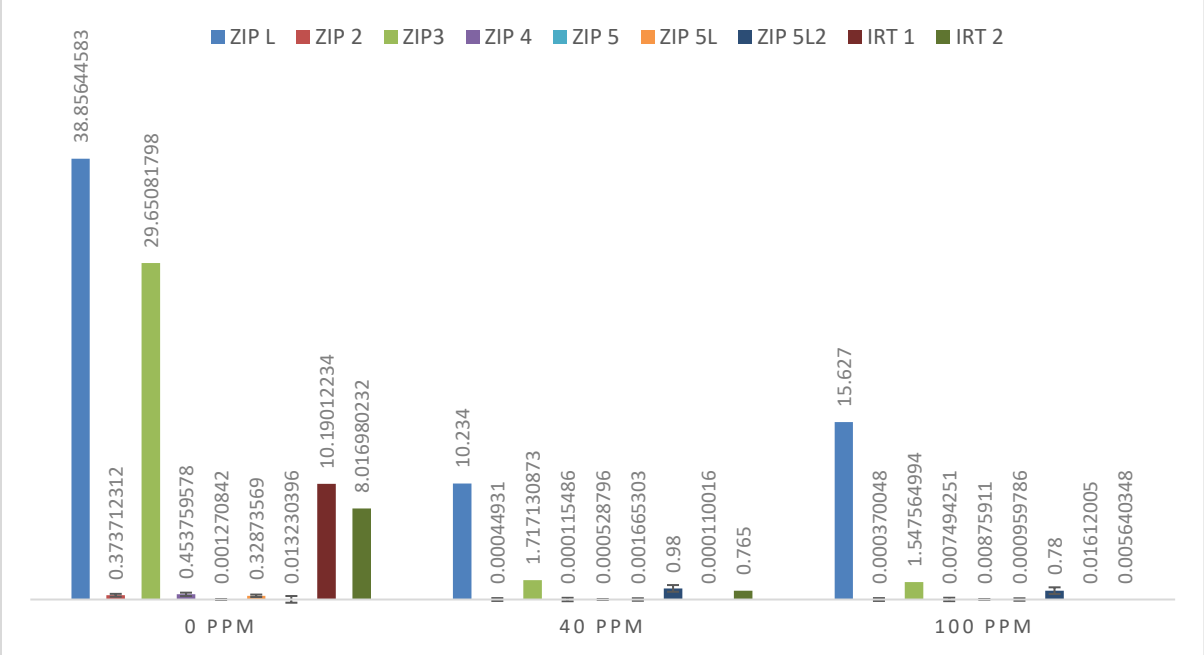

Figure 8. Relative profiling of Different Zinc Transporter genes transcripts in leaf In Genotype NTH-242 at 0, 40, 100 PPM of Zinc levels at flowering stage. Relative mRNA abundance of each gene was normalized with SLUBI gene. Data from RT-PCR was analysed according to $2^{-\Delta\Delta Ct}$ method. Error bars indicate standard deviation**.**

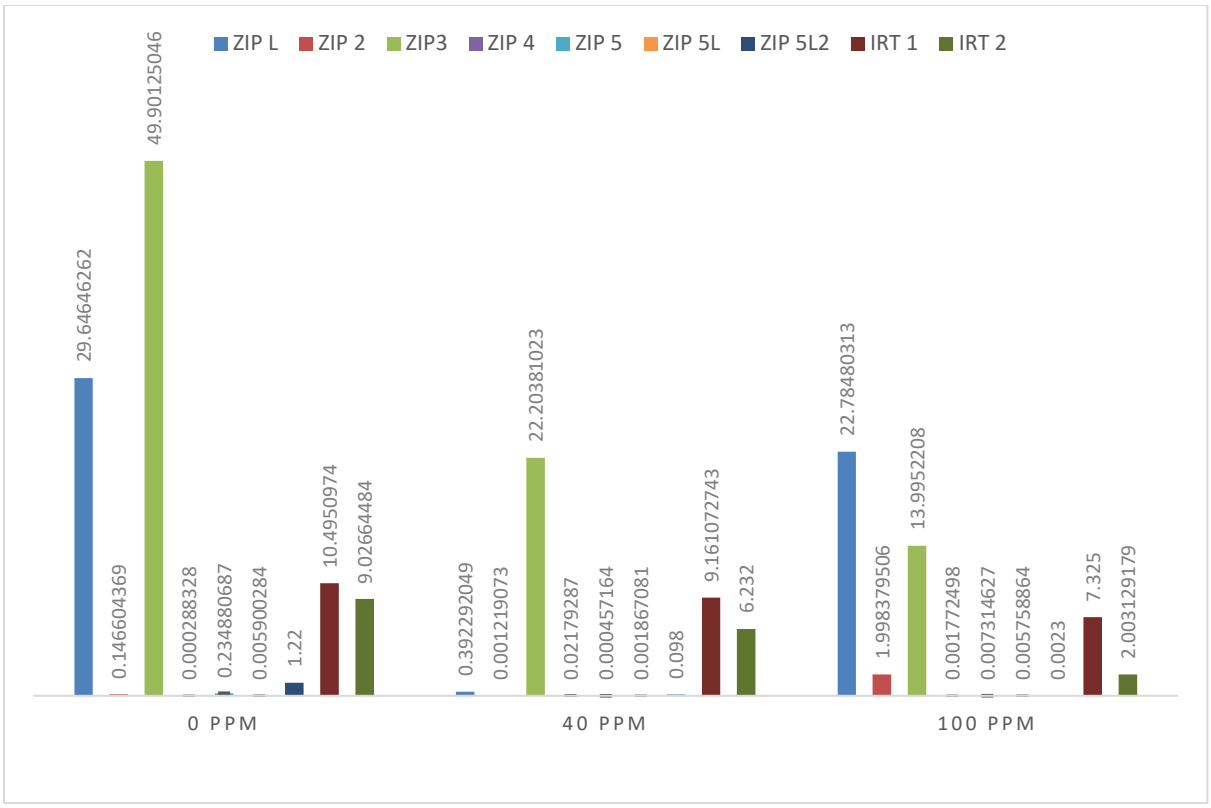

Figure 9. Relative profiling of Different Zinc Transporter genes transcripts in leaf In Genotype LA-2662 at 0, 40, 100 PPM of Zinc levels at flowering stage. Relative mRNA abundance of each gene was normalized with SLUBI gene. Data from RT-PCR was analysed according to $2^{-\Delta\Delta Ct}$ method. Error bars indicate standard deviation**.**

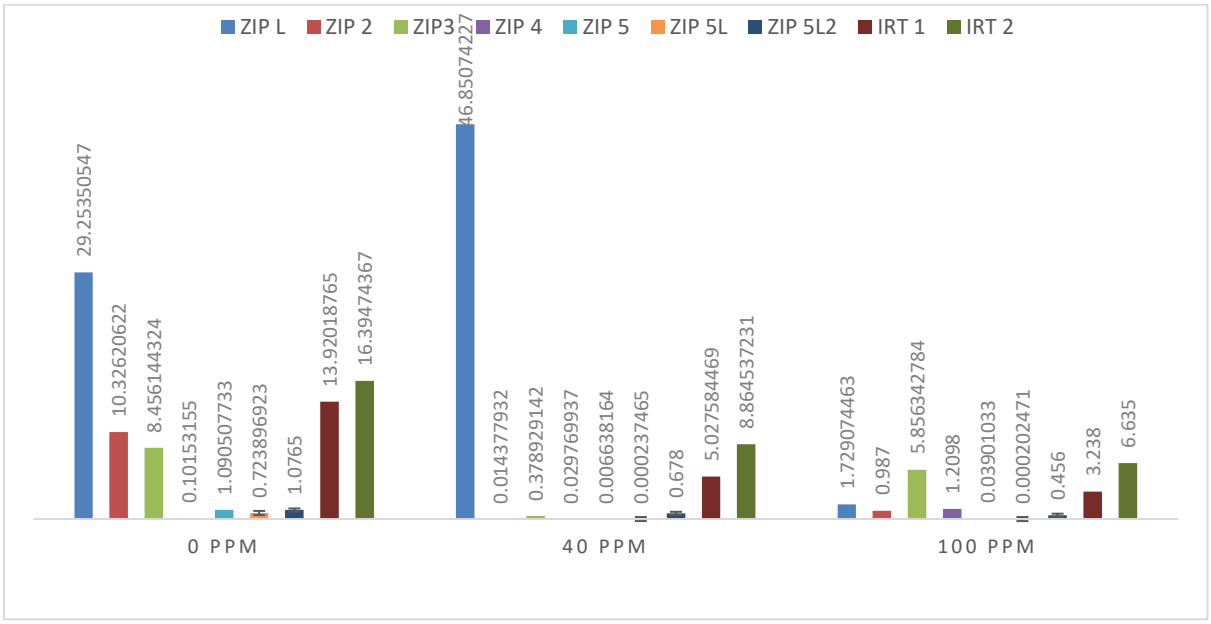

Figure 10. Relative profiling of Different Zinc Transporter genes transcripts in root In Genotype NTH-242 at 0, 40, 100 PPM of Zinc levels at flowering stage. Relative mRNA abundance of each gene was normalized with SLUBI gene. Data from RT-PCR was analysed according to $2^{-\Delta\Delta Ct}$ method. Error bars indicate standard deviation**.**

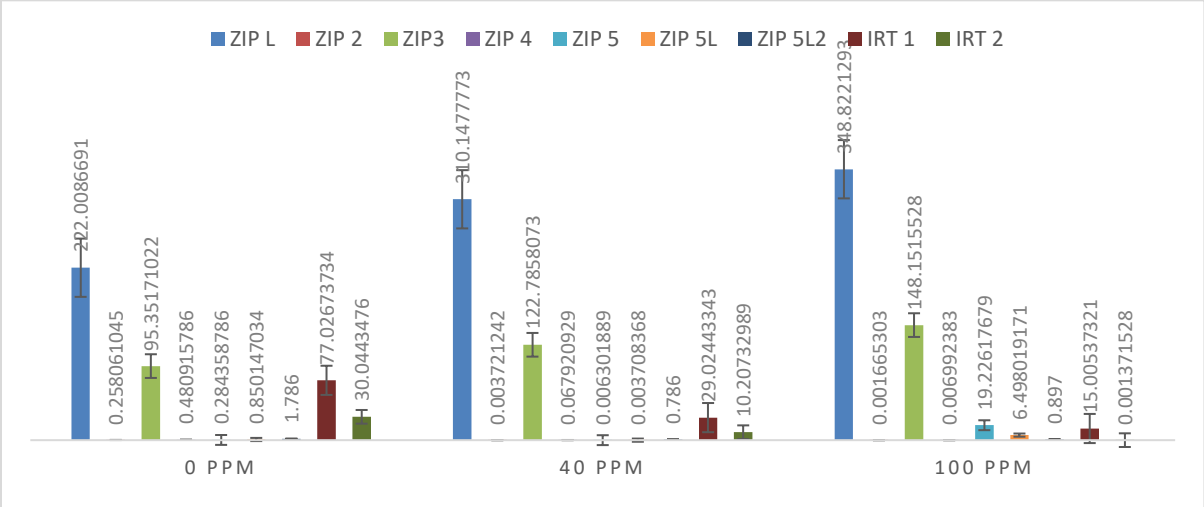

Figure 11.Relative profiling of Different Zinc Transporter genes transcripts in root In Genotype LA-2662 at 0, 40, 100 PPM of Zinc levels at maturity stage. Relative mRNA abundance of each gene was normalized with SLUBI gene. Data from RT-PCR was analysed according to $2^{-\Delta\Delta Ct}$ method. Error bars indicate standard deviation**.**

In LA-2662 genotype, under controlled conditions only SLZIP3 gene transcripts was observed and then its transcription decreases in other levels of zinc. While at 100 PPM level of zinc, accumulation of transcript of gene SLZIPL and SLZIP3 was higher in leaves as compared to other ZIPS genes at vegetative stage as compared to 40 PPM level. This showed that SLZIP3 transcript was sensitive to Zn environment in leaf in both genotypes. LeIRT1 showed approximately same transcription pattern at all level of zinc while LeIRT2 showed high transcription at low zinc level and decreases its transcription as zinc level increases. (Fig. 9).

***Transcription profiling pattern of SLZIP gene in root tissue of tomato plant at vegetative stage*:** A varying pattern of SLZIPL gene expression was observed in root tissue under different Zn level in NTH-242 genotype. The transcription pattern of SLZIPL gene was two times higher at 40 PPM zinc concentration as compared to control and little transcription at 100 PPM. The accumulation of gene transcript SLZIP2 was also up-regulated under low zinc concentration. Iron regulated gene LeIRT1 also showed transcription pattern in root tissue under controlled conditions, while its transcription pattern was two times higher under Zn deficient environment as compared to 40 PPM and 100 PPM level and LeIRT2 gene transcription pattern was also higher under Zn deficient environment and decreased as the level of zinc increased. In root tissues, the transcription pattern of SLZIP4,5,5L,5L2 showed little transcription at 100 PPM and very low transcription was observed in control and at 40 PPM zinc level (Fig. 10).

In LA-2662 genotypes different transcription pattern of targeted gene was observed as in NTH-242 genotype. The accumulation of gene transcript SLZIPL and of SLZIP3 was up-regulated at 100 PPM level of zinc and their transcription level was low at 40 PPM and 0PPM and two iron regulated gene LeIRT1 and LeIRT2 were up-regulated as the zinc concentration decreased in soil. The transcription pattern of SLZIP2,4,5L,5L2 showed same transcription level at all zinc levels. It means their transcription pattern was not affected by the zinc concentration (Fig. 11).

DISCUSSION

The use of micronutrients as a fertilizer, along with the use of major nutrient fertilizer (NPK), has got major effect on crop yield and crop quality. Crops cultivated on micronutrient deficient soils affects crop productivity in the same fashion as crops grown on macronutrient deficient soils. With increase in the demand of vegetable crops by health conscious people, there is need to provide balanced fertilizer of both macro and micronutrients to crops (Kumar *et al*., 2016).

The ZIP transporter gene family is found in many organisms at all phylogenetic level i.e. fungi, bacteria, insects, plants and mammals that helps in up taking different metals in these organsims but the mechanism of their regulation in organisms is still unknown except in yeast. (Zhao *et al*., 1998).

One of the major Zn transporter family is ZIP (ZRT-IRT like protein) (Sinclair and Kramer, 2012). This family comprises of 15 members in *A. thaliana*. The mutant of *A. thaliana* to IRT accumulate less zinc as compared to wild type, indicating its role in Zn transport (Henriques *et al*., 2002). Recently, it was reported that ZIP genes in Arabidopsis and rice play important role in transporting the Fe and Zn through

functional analysis (Lee and An, 2009; Lee *et al*., 2010). Studies on expression level in *A. thaliana* roots, showed that ZIP genes like ZIP1, ZIP3 and ZIP4 are expressed under Zn deficient condition while ZIP1, ZIP3, ZIP4, ZIP9 and ZIP10 are overexpressed in Zn responsive species like *A. halleri* as compared to A. thaliana (Talke *et al*., 2006).

The transcription profiling of different SLZIP genes under different zinc status at various stages in leaf and root tissues of two tomato genotypes i.e. one is the highest Zn accumulator LA-2662 and other is the lowest Zn accumulator NTH-242 genotype. RT-PCR results showed that transcription of genes SLZIP3 and SLZIPL in leaves and roots at vegetative stage is upregulated in Zn deficient environment and their transcription is down regulated as Zn concentration increases in the growth medium and the transcription of these genes were low in genotype NTH-242. While at maturity stage mostly SLZIP3 and SLZIPL gene showed their high transcription pattern under zinc deficient environment in genotype LA-2662. Iron regulated gene LeIRT1 and LeIRT2 genes showed their similar transcription pattern in both genotypes at vegetative stage while at maturity stage in both leaf and root tissues their transcription pattern increases under Zn deficient environment and decreases as Zn concentration increases in the growth medium of tomato plant. As reported in *Arabidopsis*, 15 ZIP genes were characterized as zinc transporter genes in roots and shoots cells and most of them are active under Zn deficient environment (Van de Mortel *et al*., 2006). Whereas results of transcript profiling in *Arabidopsis* discovered a set of 15 genes that contain ZDRE (zinc deficiency responsive element) motif in the upstream region of the gene which induced in response to zinc deficiency (Assuncao *et al*., 2010). In other studies of expression analysis in tomato plant, quantitative transcription of SLZIPL and SLZIP5 was observed in Zn sufficient and deficient condition. While gene SLZIP3, SLZIPL mostly showed their transcription under zinc deficient environment and the transcription of all SLZIPs gene studied were found less under toxic condition of zinc (Pavithra *et al*., 2016).

***Conclusion*:** Using the transcriptome analysis, we identified two genes SLZIP3 and SLZIPL that upregulated in genotype LA-2662 under Zn deficient environment and it might help in more Zn accumulation in this genotype. By increasing the expression of these two genes in LA-2662 genotype we can made it Zn fortified tomato crop that helps in treating malnutrition problems prevailing in human beings especially in children.

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