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TIR-NBS-LRR genes play a role in plant defence against biotic stress in *Solanum lycopersicum*

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Abstract: Among the many biotic factors with adverse effects on *Solanum lycopersicum* (tomato), diseases caused by fungi, viruses and nematodes are notable. Since the genome of *S. lycopersicum* became available, efforts have continued to identify the genes and proteins associated with the plant defence activity. One such gene family belongs to TIR-NBS--LRR (TNL), a subfamily of larger NBS-LRR genes. In total, 27 full-length TNLs were identified via genome wide analysis. Four pairs of segmental duplication events were observed involving different pairs of chromosomes, except the pairing of *Solyc02g082050-Solyc02g032650*, which were both present on chromosome 2. More than twenty nine percent (29.63%) of the genes were localised on chromosome 1 alone. Hormone-mediated biotic stress-responsive cis-regulatory elements were detected for methyl-jasmonate, salicylic acid (TCA motif) and ethylene (ERE motif). Differential gene expression was observed for many genes under different plant tissues and biotic stresses. The upregulation of many genes including *SlBS4* was observed against *Alternaria solani* attacks in the disease tolerant varieties. Altogether, the results suggested that TNLs play a significant role in plant defence under biotic stress.

Keywords: *Solanum lycopersicum;* TIR-NBS-LRR genes; biotic stress; early blight disease

Tomato is a major horticulture crop and a rich source of antioxidants and vitamins. Early blight, late blight, bacterial spot, bacterial wilt, and Fusarium wilt are among the bacterial and fungal diseases that damage tomato plants. Early blight is more prevalent among these diseases in areas with more dew, rain, and humidity. Defoliation is the most serious stage of early blight disease since it affects the fruit quality and yield, resulting in a severe yield reduction (Adhikari et al. 2017). Efficient strategies for disease prophylaxis are limited due to inadequate knowledge about the interaction mechanism between the host (tomato) and pathogen *Alternaria solani*. Therefore, understanding defence-associated signals, and the corresponding genes and pathways need thorough attention.

Genes belonging to the NBS-LRR family, including members of the TIR-NBS-LRR (TNL) family, are considered one of the most effective defence mechanisms against pathogens (Chen et al. 2016). The NBS-LRR genes encode proteins with nucleotide binding sites (NBSs) and leucine-rich repeat (LRR) domains, often present in large numbers in the plant genome (Zhao et al. 2016). NBS-LRR genes reported in plant defence include both CC-NBS-LRR (CNL) and TNLs. The CNL members include wheat powdery mildew resist-

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ance (*Pm3b* and *Pm8*), rice blast resistance (*Pi64*) and cotton verticillium wilt resistance (*GbRVd*) as some prominent examples (Zhu et al. 2021). While, a few studies have reported TNLs in providing resistance against various biotic stress included verticillium wilt resistance (*GhDSC1*), *Diplocarpon rosae* resistance (*Rdr1*), resistance to downy mildew (*MrRPV1),* resistance to *Colletotrichum fructicola* causing Glomerella leaf spot (*MdTNL1*), resistance against phytophthora root rot (*GmTNL16*) and resistance against *Oidium heveae* (*WRR4B*) (Song et al. 2023; Zhou et al. 2022; Mei et al. 2024). In Solanaceae species, only a handful of TNL genes have been reported against pathogens. Examples include *Gro1-4* and *StTNLC7G2* in potatoes against the *Globodera rostochiensis* Ro1 pathotype and *A. solani* (Song et al. 2023), respectively. To expand the useful gene pool, many reports have identified the TNLs by genome-wide analysis. For instance, investigations on *Rosa chinensis* revealed 96 intact TNL genes, while, in potatoes, 44 genes encoding 60 TNL transcripts were identified (Song et al. 2023). Therefore, genome wide identification was frequently performed in the past (Andolfo et al. 2013, 2014; Qian et al. 2017) to find out the total NBS-LRR gene family in tomatoes, including non-TNL members like CNL. However, elaboration upon role of TNL members under biotic stress was almost negligible in such reports. A recent report on genome wide identification of NLRs (NBS-LRR receptors) identified the TNL repertoire, yet, an expression analysis was lacking for these genes (TNLs) for early blight disease as well as for other biotic stresses (Bashir et al. 2022). Thus, in the present work, after identifying the TNL gene family members, they were assessed by protein-protein interaction prediction and expression analysis using RNA-Seq data for the spatio-temporal expression and biotic stresses. The expression analysis of selected genes by real time polymerase chain reaction (PCR) projected a strong role for them under *Alternaria solani* attack in two different varieties. This will help not only in the identification of putative TNL resistance gene analogues against early blight disease, but also pave the way for their utilisation against other tomato pathogens.

MATERIAL AND METHODS

Extraction of TNL sequences and their functional prediction. Tomato (*Solanum lycopersicum* L. assembly v3.20; Heinz) protein sequences (35,768) downloaded from the Sol Genome Network (https://solgenomics.net; accessed on August 5, 2020) were used to anticipate the NBS-LRR proteins. CDD (Conserved domain database) search was applied for the TIR, NBS and LRR domain search for the whole tomato proteome (Dubey et al. 2022). The absence of a coil-coiled motif was confirmed using the COILS program (Lupas et al. 1991) with default parameters. To explore the motif variation among the identified *Solanum lycopersicum* TNLs (SlTNLs), the predicted protein sequences were submitted to MEME (version 4.12.0) (Bailey et al. 2009). The motif analysis was then performed on all 27 predicted TNL proteins. The physicochemical properties were assessed using the ProtParam tool (Gasteiger et al. 2005). The localisation of all TNL proteins was predicted by Target P and WoLF PSORT. The exon-intron were visualised using the Gene Structure Display Server [GSDS v.2.0 (http://gsds.gao-lab.org/ accessed on July 20, 2021]. The Gene Ontology (GO) enrichment was performed using ShinyGO (version 0.77) (accessed on July 10, 2023). The cis-acting elements in promoter (2 kb upstream sequence) was predicted using PlantCARE (Lescot et al. 2002).

Chromosomal localisation and phylogenetic tree construction. All the gene positions were pinned down by deploying the information outlined in ITAG3.2_gene_models.gff, accessed from the SGN data sharing site at ftp://ftp.solgenomics.net/tomato_genome/annotation/ITAG3.2_release/. Genes were graphically portrayed on the chromosome using the PhenoGram tool (Wolfe et al. 2013). Full-length sequences of all 27 TNL peptides were aligned using Clustal Omega, and alignment obtained was subjected to MEGA (version 7.0) (Kumar et al. 2016) for phylogenetic analysis using Neighbour-joining (NJ) method with 1 000 bootstraps.

Gene duplication, synteny and protein-protein interaction analysis. TNL duplication events were predicted using the MCScanX program. A dual gene synteny analysis was performed by comparing the genomes of *Solanum lycopersicum* and *Arabidopsis thaliana*. The results were visualised using TBtools (version 1.130) (Chen et al. 2020). Protein-protein interaction (PPI) prediction was performed on Cytoscape (version 3.9.1) with the stringApp plugin (https://version-11-5.string-db.org/).

Expression analysis of *SlTNL*s in different tissues and biotic interactions. The Tom Express database (http://tomexpress.toulouse.inra.fr/; ac-

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cessed on August 2, 2021) was utilised to download the normalised expression count of all the transcripts (RNA sequencing dataset) from different plant tissues under normal conditions and under biotic interaction. The available datasets for the nematode infection (*Meloidogyne javanica*) at 0, 2, 5, and 15 dpi on Avigail-870 roots, attack of *tomato yellow leaf curl virus* on CLN2777A (resistant) and TMXA48-4-0 (susceptible) cultivars for 0, 3 and 7 dpi and the Arbuscular mycorrhiza fungi (AMF) (*Funneliformis mosseae*) interaction on Money-Maker (at the breaker stage of fruit ripening) were assessed. The spatial expression of the transcripts was grouped as the seed, leaf, root, flower, and fruit. A heat-map was prepared using the R-statistical package-gPlots (RColorBrewer).

Expression analysis of TNL transcripts in tomatoes under *Alternaria solani* infection. Two frequently used tomato varieties under commercial cultivation viz. Pusa Ruby and Pusa Rohini were utilised. The fungal pathogen *Alternaria solani* was procured from MTCC, Chandigarh, India (MTCC strain 4632). The plants were grown in a controlled environment with 16 h light at 25 °C. The two-week-old tomato seedlings were spray inoculated (1×10^4 spores/mL) with *Alternaria solani.* Leaf samples were collected as a biological triplicate at 0, 1, 2, 3, 5, and 7 days post inoculation (dpi). The total RNA was isolated using the IRIS protocol and 2 µg of the total RNA was utilised for the cDNA synthesis (Dubey et al. 2022) using a high-capacity cDNA synthesis kit (Applied Biosystem, USA). The cDNA was diluted 10-fold with diethyl pyrocarbonate treated (DEPC) water. The Power Sybr Green PCR master mix (Applied Biosystem, CA, USA) was used for the gene expression study using an optical 96 well plate on an MX3000P real-time PCR machine (Agilent technologies, Germany). All the experimental run included an NTC (no template control) for the detection of any DNA contamination. The cycle condition for the reactions was 95 °C for 7 min followed by 40 cycles of 95 °C, 60 °C and 72 °C for 30 s each, followed by 65 °C for 5 s and finally 95 °C for 50 s. The elongation factor (*SlEf1a*) was selected as the reference gene based on the successful use of the same in previous reports (Kissoudis et al. 2016), though, primers were designed in-house using Primer (version 2.2.3). All the primer sequences were catalogued in Electronic supplementary material (ESM) Table S1. The relative expression was calculated by the $2^{-\Delta\Delta CT}$ methodology. Genes with a relative expression value cut-off of > 2 fold were considered induced or repressed. Tukey's post-hoc test was run to assess the significance among the differential expression data.

RESULTS

Identification of twenty-seven TIR-NBS-LRR proteins in the tomato. In total, 27 proteins with genes encoding their transcripts were identified via the Genome wide CDD search. Simpler nomenclature was also applied (ESM Table 2) following a previous report (Dubey et al. 2022). The phylogenetic analysis of the identified TNLs in the tomato showed alignment of the proteins and subgroup segregation based on the motifs (Figure 1A). The exon-intron arrangement revealed variations in the number of introns, ranging from 2 introns in *SlTNLC1G5* to 10 introns in *SlTNLC1G7*. One gene, *SlTNLC8G2*, was found to be intron-less (Figure 1B). The physical mapping of TNL genes on the tomato chromosomes reflected the presence of 8, 3, 2, 3, 3, 1, 2 and 5 genes on chromosome 1, 2, 4, 5, 7, 8, 9 and 11, respectively (Figure 2A). Chromosome 1 carries the greatest number of TNL genes (29.63%) while no gene was mapped on chromosome 3, 6, 10 and 12. Two proteins, SlTNLC1G7P and SlTNLC11G2P, were detected with the tandem repeat of all three domains (repeat of TIR/NBS/LRR within the protein), making their length more than 2 000 amino acids (ESM Figure 1). The presence of all four conserved TIR-type motifs, viz., TIR-1, TIR-2, TIR-3 and TIR-4, was confirmed (ESM Figure 2A). In our analysis, we identified the TIR-1, TIR-2, TIR-3, and TIR-4 motifs, of which only three (TIR-1, TIR-2, and TIR-3) were previously reported in other Solanaceae members (Lozano et al. 2012). Eight major motifs (Kinase-2, RNBS-A, P-loop, RNBS-B, RNBS-D, RNBS-C, MHDV, and GLPL) were observed in the NBS domain. A slight difference was observed in the MHDV motif, where the valine (V) amino acid was replaced by leucine (L), resulting in the MHDL motif.

Physico-chemical properties and gene ontology of the identified proteins. The minimum protein length of 384 amino acids and molecular weight of 44.21608 kDa was observed for SlTNLC7G2P, while the maximum length of 2 871 amino acids and molecular weight of 325.86764 kDa was observed for SlTNLC11G2. The length of twenty

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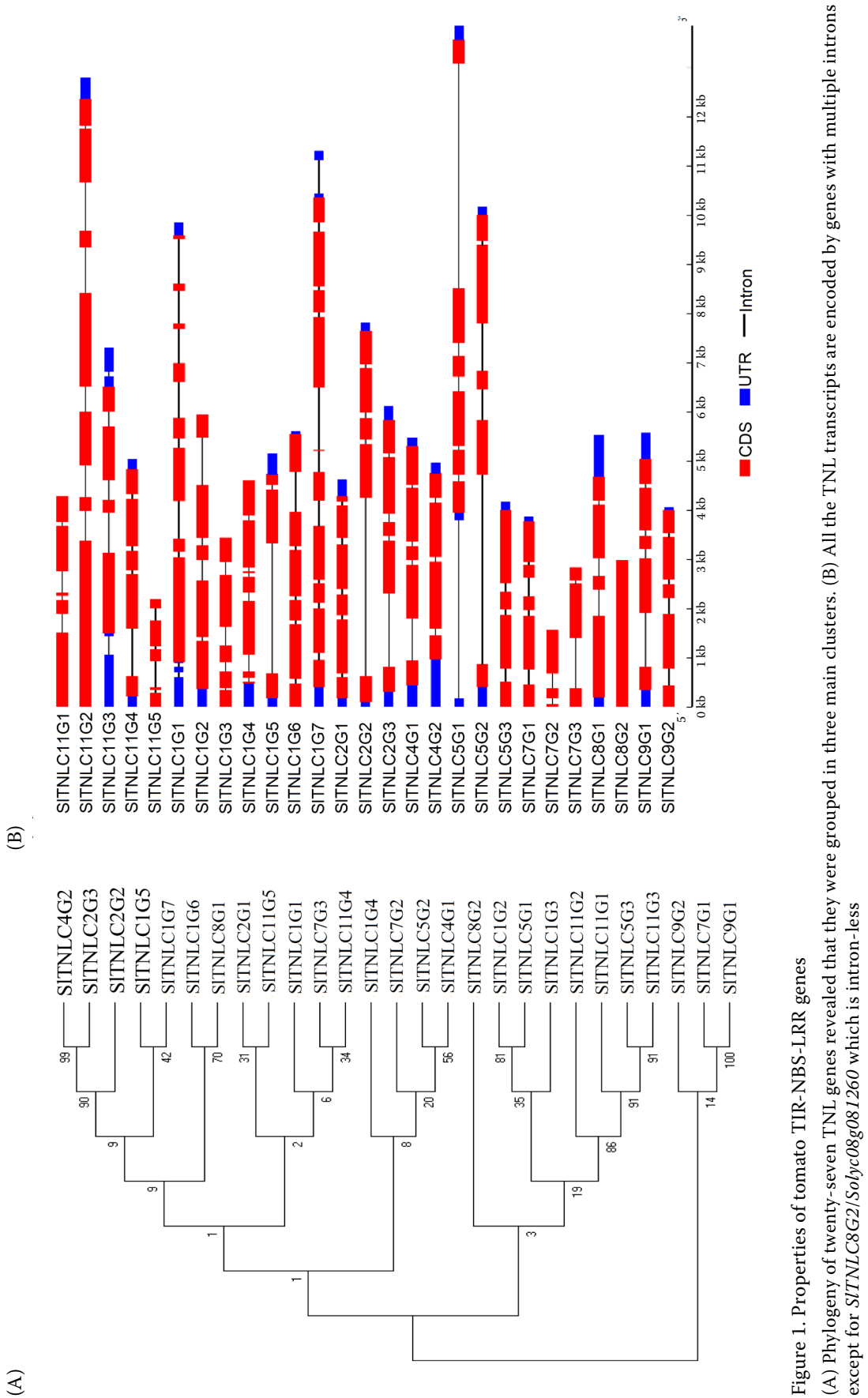

Figure 1. Properties of tomato TIR-NBS-LRR genes

(A) Phylogeny of twenty-seven TNL genes revealed that they were grouped in three main clusters. (B) All the TNL transcripts are encoded by genes with multiple introns except for *SlTNLC8G2*/*Solyc08g081260* which is intron-less

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proteins exceeded one thousand amino acids, implying a family with bulky proteins. The isoelectric point (pI) projections were in the range of 5.43 (SlTNLC11G2P) to 9.37 (SlTNLC8G2). The GRAVY index (a measure of hydropathy) of the SlTNLs showed that they are mostly hydrophilic and sol-

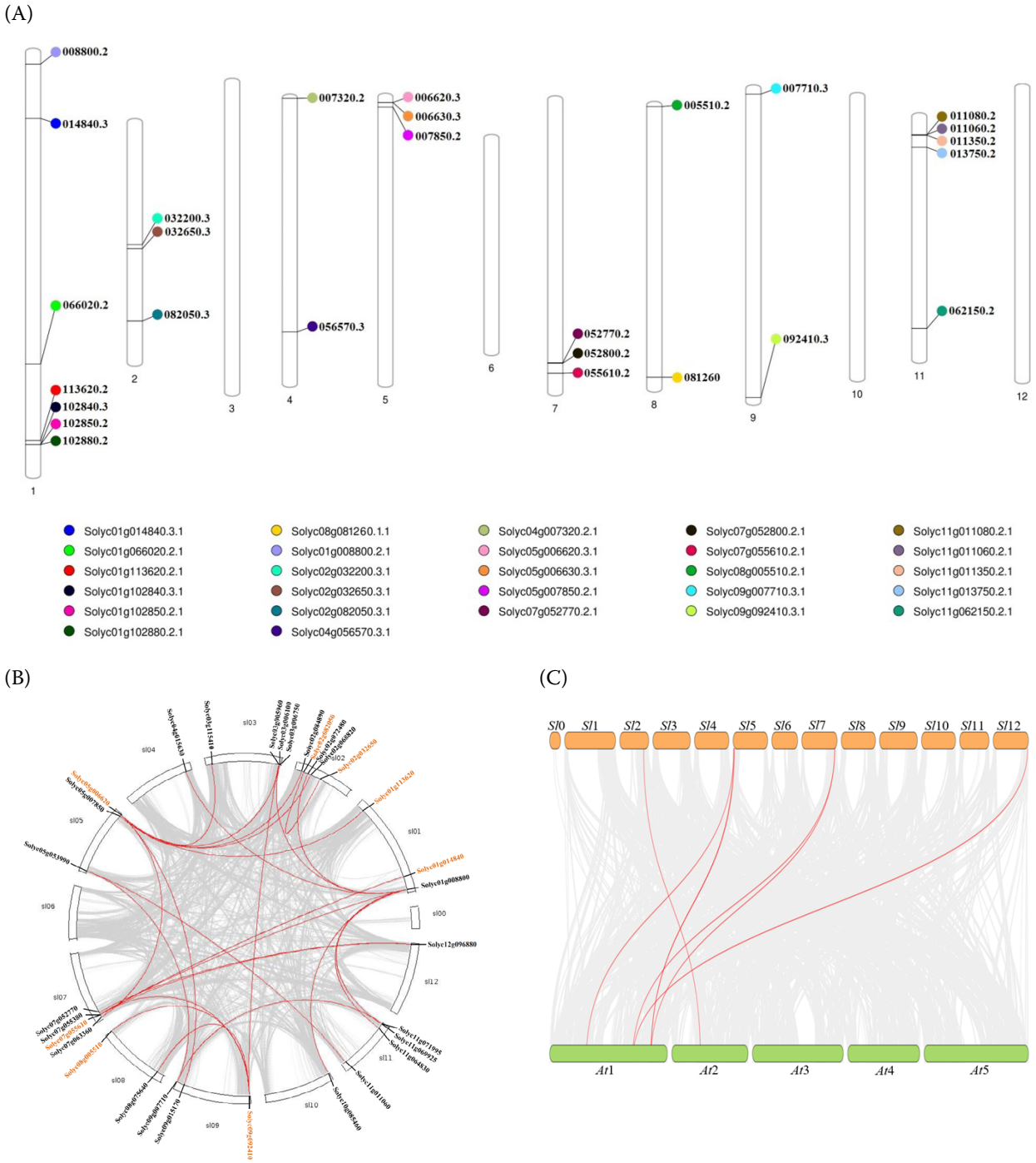

Figure 2. (A) All the genes were mapped on the different chromosomes of the tomato. There were no TNL genes present on chromosome 3, 6, 10 and 12. Two small cluster of TNLs were observed on chromosome 1 and 11, consisting of 3 genes each. (B) Duplication analysis suggested mostly the segmental duplication of TNL genes. A total of 4 pairs of TNL-TNL duplication were observed (highlighted by the orange font). The other duplication events included the genes belonging to different resistance gene classes. (C) The tomato to *Arabidopsis* synteny was contributed by three sets of TNL genes. Most of the TNLs identified by synteny were located on chromosome 1 of *Arabidopsis* except one on chromosome 2

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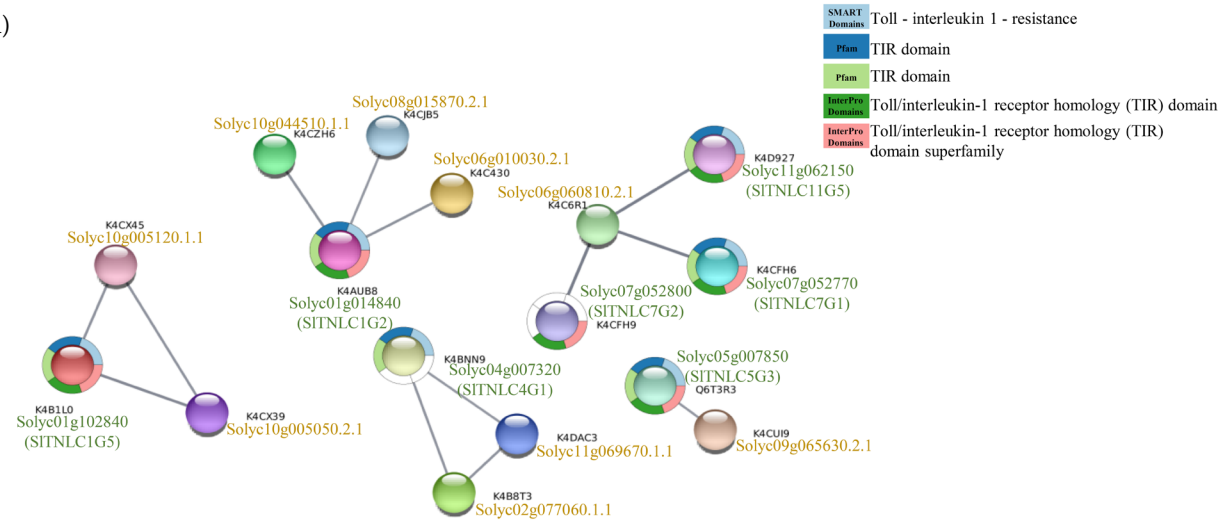
uble as all the proteins predicted with value < 0. *In silico* localisation analysis predicted that 13 proteins would be located in the nucleus and six each in the cytoplasm and chloroplast. One protein for each also showed putative localisation in the vacuole and plasma membrane (ESM Table 2). The gene ontology (GO) analysis revealed the enrichment of the GO terms in relation to the signal transduction, signalling, cellular response to stimulus, hydrolase activity and plant defence response for all the TNL proteins identified here (ESM Figure 2B).

Segmental duplication events observed. Across the tomato genome, there were 4 pairs of TNL-TNL segmental duplication events contributed by 8 TNLs (ESM Table 3). While duplication events of TNL vs other genes identified 22 pairs. Among them, *Solyc01g008800* was found to be involved in 7 TNL-all events with pairing to *Solyc02g072480*, *Solyc03g006100*, *Solyc04g015630*, *Solyc07g055380*, *Solyc10g085460*, *Solyc11g064830*, and *Solyc11g069925* in the genome. The duplication event mostly identified the genes like CNL and receptor like kinases (RLKs) (Figure 2B). The collinearity was observed in the TNL to the truncated TIR domain containing genes, too.

TNL genes showed synteny with *Arabidopsis*. Gene collinearity was explored between *Arabidopsis thaliana* (At) and *Solanum lyco-*

(A)



(B)

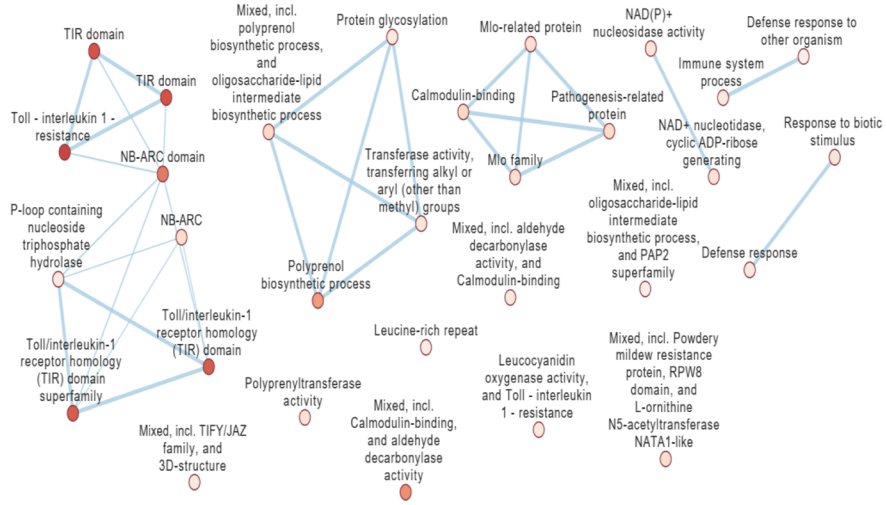


Figure 3. (A) Seven TNL proteins were found with significant interaction for the protein-protein interaction prediction (B) Enrichment of defence associated terms like response to biotic stimuli, the MLO-related protein, powdery mildew resistance protein RPW8 and pathogenesis related protein

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***persicum* (Sl) genomes.** Gene *Solyc02g082050.3.1* (T-N-L) present on chromosome 2 of *Solanum lycopersicum* shared collinearity with *AT2G17050.1* (N-L-T) on the *A. thaliana* chromosome 2 (Figure 2C). This pairing shared NBS-LRR traits, distinguished by differing TIR domain positions, potentially influenced by the genetic milieu. Similarly, *Solyc05g007850.2.1* and *Solyc05g006620.3.1*, present on chromosome 5, showed synteny to *AT1G69550.1* and *AT1G27170.2* on chromosome 1 of *A. thaliana*. Their common TNL domains hinted at conserved defence roles within both plants. A noteworthy collinear gene pair was observed, where tomato gene *Solyc07g055380.1.1* (NBS-LRR-C_JID), located on chromosome 7, was devoid of the TIR domain, yet, paired with *AT1G69550.1* (TIR-NBS-LRR-C_JID) situated on *A. thaliana*'s chromosome 1 (ESM Table 4). The lack of the TIR domain in *Solyc07g055380.1.1* could be attributed to the divergence between the two plant species, potentially resulting in the loss of the TIR domain during evolutionary adaptation.

Many stress-responsive cis-regulatory elements and defence-associated interacting partners detected. Major hormone-responsive regulatory elements, such as salicylic acid (SA-TCA motif), abscisic acid responsive element (ABRE), methyl jasmonate (MeJA), and ethylene responsive elements (EREs) were found in the promoter region of many genes. The upstream region of 15 TNLs carried SA-responsive elements; 16 TNLs were found to have ABRE elements, while the promotors of 8 genes carried MeJA-responsive elements (ESM Figure 3). ERE was predicted in the upstream sequence of 9 genes. The defence responsive elements like W-box and the Wun motif were detected in the promotors of 9 and 7 genes, respectively, while TC-rich repeats were detected in 5 genes. The upstream region of *Solyc04g007320.2* and *Solyc11g062150.2* showed the presence of TCA elements seven and six times, respectively, indicating the high chance of being governed by them. Only a handful of TNLs were detected for the protein-protein interaction with a stringent cut off value of 0.7 taken to consider any interaction prediction as being meaningful. In Figure 3A, these proteins and their interacting partners were denoted by UniProt identifiers and locus IDs. The largest number of interacting partners were predicted for Solyc01g014840, with two interacting partners identified each for Solyc01g102840 and Solyc04g007320. The enrichment analysis showed a network of terms, mostly associated with the biotic stress response, signal transduction and plant defence (Figure 3B). The visualisation underscores the pivotal role of TIR domains in providing resistance while emphasising their seamless interaction with plant immunity-associated functional terms.

Gene expression analysis in different plant tissues and biotic interactions. The normalised expression count of all the TNL transcripts, as available in the TomExpress database and resulting heatmap, showed that they expressed differentially in different plant parts. Based on the obtained heatmap, only a few genes showed dormant expression across all the plant parts including *Solyc01g014840*, *Solyc11g011060*, *Solyc11g062150*, *Solyc01g102850*, and *Solyc07g052770* (Figure 4A).

Two genes, *Solyc07g055610* and *Solyc09g092410*, showed near constant expression across all the samples, but were found induced in the root samples. *Solyc11g011350* was the only transcript showing significant induction in the seed samples. Four transcripts, *Solyc11g013750*, *Solyc05g006620*, *Solyc01g102840* and *Solyc11g011080,* showed partial induction in the fruit transcriptome datasets at different stages while being partially repressed in the vegetative parts of the leaf and meristem. Two transcripts, *Solyc01g113620* and *Solyc01g102880* showed exclusive induction in all the leaf samples that were probably involved in the basal defence against leaf-borne pathogens.

Among the biotic stresses, nematode attacks could be a major problem for tomato plants. Figure 4B showed that expression of nine genes under a *Meloidogyne javanica* attack were downregulated. Most of them were significantly downregulated after the successful colonisation at 15 dpi. Twelve genes did not show any change in expression. While three genes, *Solyc02g032200*, *Solyc07g055610* and *Solyc01g014840* were induced after the *M. javanica* attack at 5 and 15 dpi. The three genes can be speculated to function in two ways, either they are acting as a susceptibility factor facilitating nematode colonisation or as part of a major defence mechanism to counter the *M. javanica*, though unsuccessfully. None of the TNL genes showed any significant induction or repression in the 3–7 dpi samples under the TYLCV attack in the resistant variety-CLN2777A. Albeit, *Solyc11g062150* showed induction in the susceptible variety (TMXA48-4-0) post-infection. *Solyc11g062150* was probably ma-

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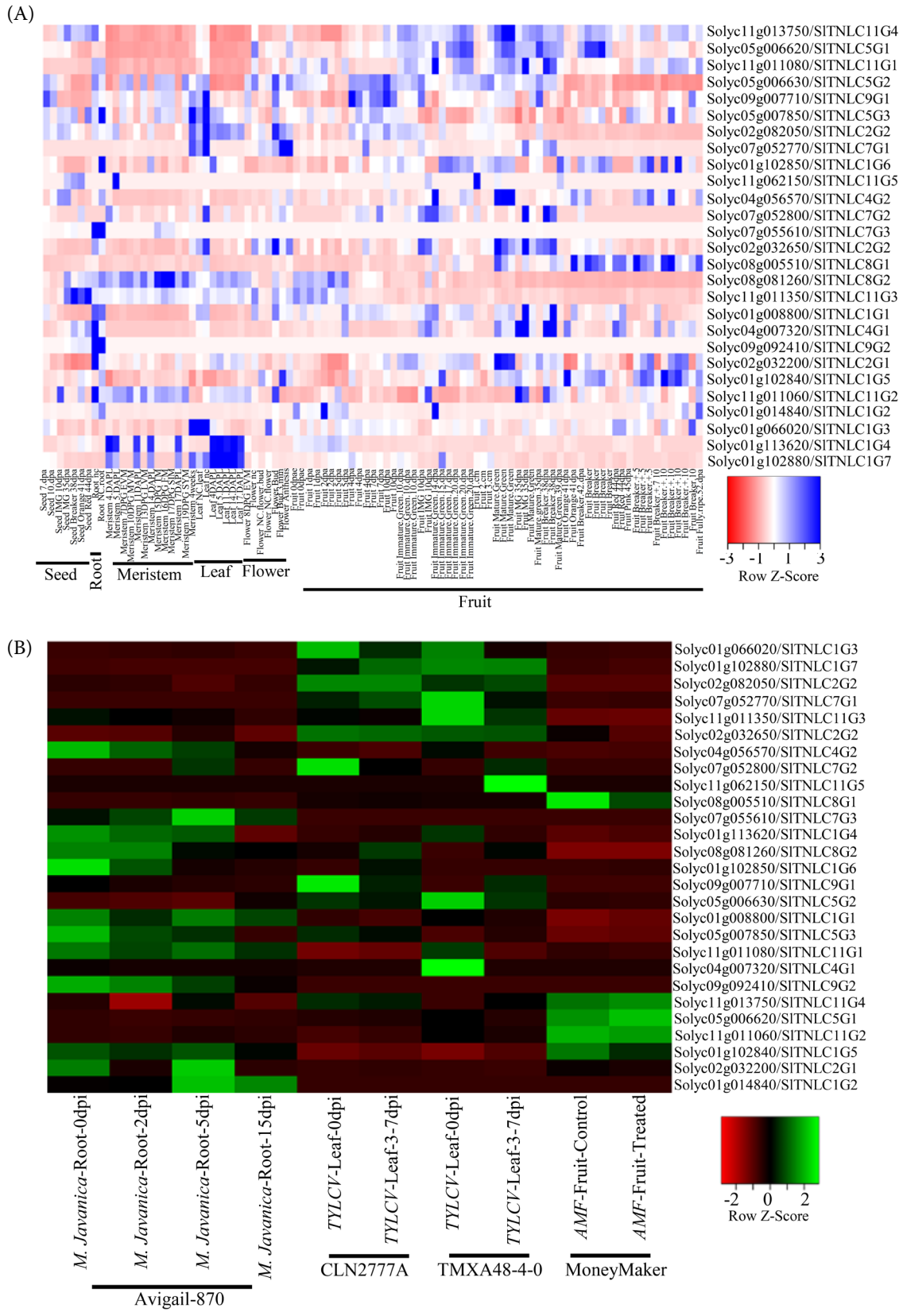

Figure 4. Gene expression analysis of all the SlTNLs reported in the study for (A) the spatio-temporal expression under different plant tissues (B) under different biotic interactions, including viral and nematode attack

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nipulated by the virus for infection in the susceptible varieties. AMF fungi did not perturb the expression of TNLs compared to the control, except for *Solyc08g005510*, where the transcript was partially downregulated.

Genes behave differentially in two varieties under early blight attack. To get an idea on the similarities and differences in the expression pattern of selected genes under different gene pools, more than one variety was chosen. Pusa Rohini is considered partially susceptible relative to Pusa Ruby. The transcript expression of *Solyc11g011350*, *Solyc08g081260* and *SlBS4/Solyc05g007850* showed an identical pattern in both varieties (Figure 5). These genes were induced early at 1 dpi with a further increase in expression up to 4–10 fold at 7 dpi. *Solyc08g005510* also followed a somewhat similar expression pattern, but with a high reduction in the expression value in Pusa Rohini after 2 dpi (Figure 5, ESM Table 5). *Solyc11g011060* expressed at a > 4 fold induction at all the time points in Pusa Rohini, while the opposite behaviour was obtained for *Solyc01g066020* which showed similar upregulation in Pusa Ruby. The differences in the expression pattern of the same gene in two varieties suggested the upstream genotype regulation of these genes governing their expression behaviour.

DISCUSSION

The tomato is the second most cultivated horticulture crop after the potato. The production of tomatoes is hindered by many pathogens including early blight disease caused by *A. solani*. A desirable gene pool is needed to enhance the disease tolerance in widely cultivated varieties. Members of the gene family NBS-LRR are one such prospect. Among the reported studies, more work has been carried out for the CNL sub-class compared to TNLs (Dubey &

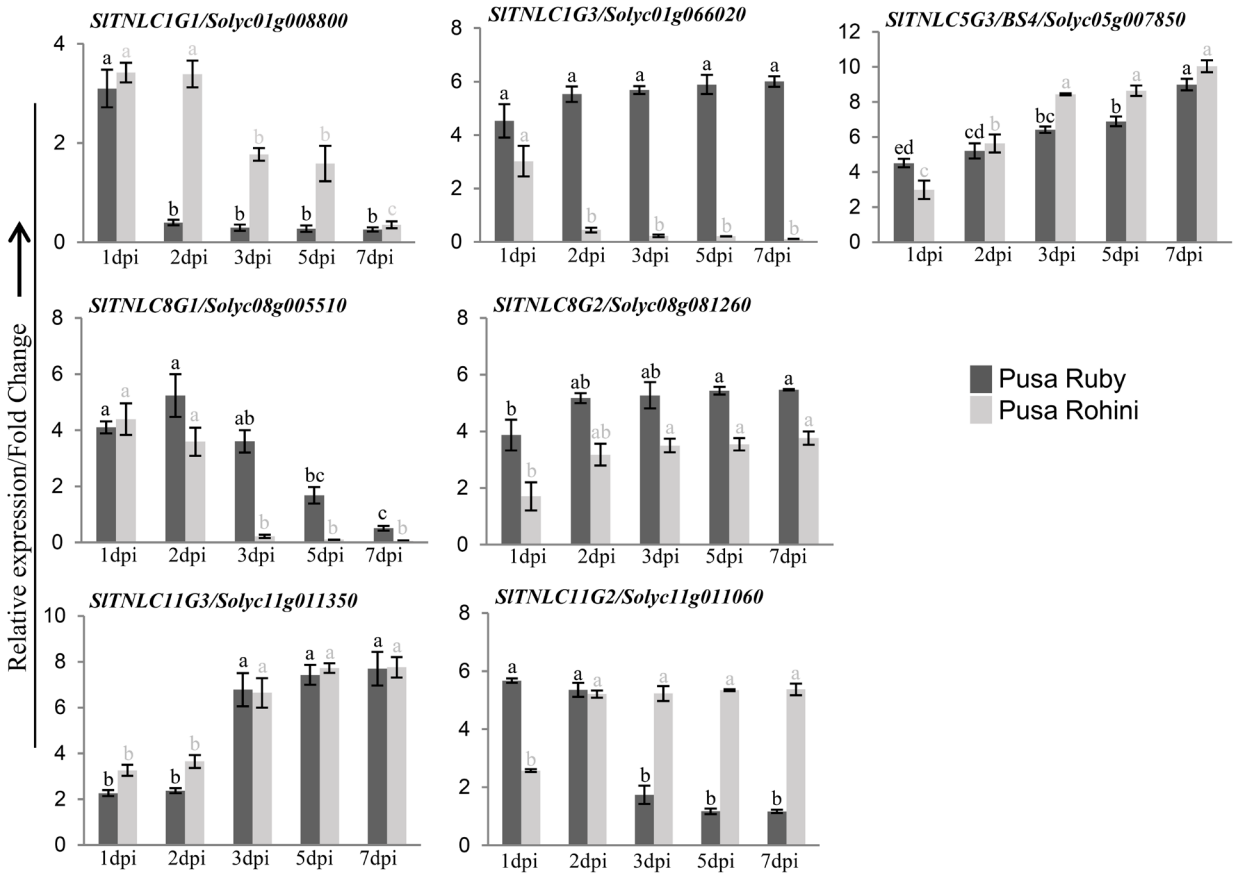

Figure 5. QRT-PCR based expression pattern of seven TNL transcripts showed their involvement in biotic stress under an *Alternaria solani* infection

Significance was based on Tukey's test. Each bar represents the standard error of the mean

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Singh 2018). For instance, *Prf* and *ph-3*, both being CNLs, were reported to be involved in providing resistance against *Pseudomonas syringae* and *Phytophthora infestans* in the cultivated and wild type tomato, respectively (Salmeron et al. 1996; Zhang et al. 2014). So far, only one TNL, *SlBs4* has been characterised extensively in tomatoes. *SlBs4* provides resistance against *Xanthomonas* sp. (Ballvora et al. 2001). In other members of Solanaceae, a few reports include the *N* gene of tobacco and *StTNLC7G2* of the potato (Dubey et al. 2022). In *Brassica nigra*, a single locus, PEK, contains a cluster of *TNL* genes and controls the butterfly egg-killing hypersensitive response, shedding light on the role of TNL receptors in insect-induced plant defence mechanisms (Bassetti et al. 2023). A duplicated TNL gene in winter oilseed rape offers promise for clubroot resistance (Kopec et al. 2021). In *A. thaliana*, many TN and TNL genes have been suggested to have a role in disease resistance. A recent study reported that the *AtSNC1* (a TNL) gene interacts with the related TNLs via TIR domains, influencing the defence related gene expression (Yeon et al. 2023). This underscores their significance in cross talk and defence signalling. Therefore, more efforts are needed to find out the role of many available TNLs with regards to tomato plant immunity.

There was very high discrepancy and fluctuation among the reported literature studies for the number of TNLs present in tomatoes. For example, Qian et al. (2017), Andolfo et al. (2013, 2014) and Bashir et al. (2022) reported different numbers for full length TNLs in tomatoes with the maximum count reaching twenty-six. This discrepancy perhaps arose due to the nature of the work being carried out with more emphasis on the *in-silico* genome wide identification of all the NBS-LRRs and less focus was placed on the TNLs. The numbers of full-length TNLs with all three domains, viz., TIR, NBS, and LRR, reported in our work can be taken with the most confidence, as all the domains were confirmed manually after the genome wide search with the maximum number being twenty-seven. TNL genes showed an average of 5 exons, yet, no instance of alternative transcription was predicted, as only 27 full length proteins could be identified against the same number of genes; unlike in *Solanum tuberosum*, where many alternate splicing events have been observed previously (Dubey et al. 2022).

The expression analysis of *SlBS4* (*Solyc05g007850*), along with six other transcripts indicated their upregulation in one of or in both varieties. As suspected, the difference in the gene pool for the two tested varieties resulted in a difference in the comparable expression pattern. Pusa Ruby, being released as more tolerant (Kumar & Kumar 2019), showed higher expression for all the genes except *Solyc01g008800* and *Solyc11g011060* against early blight disease. Even *Solyc11g011060* induces more than a 4-fold increase up to 2 dpi in Pusa Ruby and a decrease in the expression afterwards. Even though the expression pattern was similar in both varieties for other genes, Pusa Ruby showed higher values most of the time suggesting regulation of the TNL members in quantitative regulation. The results validate the less tolerant behaviour of Pusa Rohini. Furthermore, the expression results of these genes could be used in the preliminary screening of any released tomato variety or plant towards susceptibility or tolerance. The role of a few genes among the tested varieties was previously reported under other biotic stresses and provides further clues for their functionality. For example, *Solyc11g011350* and *SlBS4* expressed in a similar pattern with both being close to the homologue of the tobacco *'N'* gene. The *'N'* gene is widely known for imparting resistance against Tobacco mosaic virus (Ikeda et al. 2021). Similarly, *Solyc01g066020* has been reported to provide defence against nematode attacks via mi-RNA modulation (Zhao et al. 2015). An MiRNA mediated plant defence response involving *Solyc01g008800* was reported against potato virus Y with an increased production of phasiRNA (Prigigallo et al. 2019).

CONCLUSION

Our study represents a significant advancement in the understanding of TNL genes in tomatoes. A rigorous analysis with manual curation confirmed the presence of full length TNL genes. Chromosome 1 carries the greatest number of TNLs suggesting their vital involvement in tomato immunity. The protein-protein interaction prediction and network analysis of these TNL proteins showed the heavy enrichment of defence associated terms, further indicating their role in plant immunity. TNLs were also revealed to be involved in the regulation of tomato development with differential spatio-temporal expression. The expression analysis based on the transcriptome data and QRT-

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PCR confirmed the strong involvement in plant defence with many transcripts getting upregulated under biotic stresses including early blight. Therefore, these TIR-NBS-LRR genes could be potential targets for gene pool introgression against biotic stress causing factors.

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