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Original article

Comparative transcriptome profiling of pomegranate genotypes having resistance and susceptible reaction to *Xanthomonas axonopodis* pv. *punicae*

Nripendra Vikram Singh [a,*], Shilpa Parashuram [a], Jyotsana Sharma [a], Roopa Sowjanya Potlannagari [a], Dhinesh Babu Karuppannan [a], Ram Krishna Pal [a], Prakash Patil [a], Dhananjay M. Mundewadikar [a], Vipul R. Sangnure [a], P.V. Parvati Sai Arun [b], Naresh V.R. Mutha [c], Bipin Kumar [c], Abhishek Tripathi [c], Sathish Kumar Peddamma [c], Harish Kothandaraman [c], Sailu Yellaboina [c], Dushyant Singh Baghel [c], Umesh K. Reddy [d]

[a] *ICAR-National Research Centre on Pomegranate, Solapur, Maharashtra 413255, India*
[b] *Chaitanya Bharati Institute of Technology, Hyderabad, Telangana 50075, India*
[c] *Nucleome Informatics Private Limited., Hyderabad, Telangana State 500049, India*
[d] *Gus R. Douglass Institute and Department of Biology, West Virginia State University, Institute, WV, USA*

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ABSTRACT

Pomegranate (*Punica granatum* L.) is an important fruit crop, rich in fiber, vitamins, antioxidants, minerals and source of different biologically active compounds. The bacterial blight caused by *Xanthomonas axonopodis*pv. *punicae* is a serious threat to the crop leading to 60–80% yield loss under epiphytotic conditions. In this work, we have generated comparative transcriptome profile to mark the gene expression signatures during resistance and susceptible interactions. We analyzed leaf and fruits samples of moderately resistant genotype (IC 524207) and susceptible variety (Bhagawa) of pomegranate at three progressive infection stages upon inoculation with the pathogen. RNA-Seq with the Illumina HiSeq 2500 platform revealed 1,88,337 non-redundant (nr) transcript sequences from raw sequencing data, for a total of 34,626 unigenes with size >2 kb. Moreover, 85.3% unigenes were annotated in at least one of the seven databases examined. Comparative analysis of gene-expression signatures in resistant and susceptible varieties showed that the genes known to be involved in defense mechanism in plants were up-regulated in resistant variety. Gene Ontology (GO) analysis successfully annotated 90,485 pomegranate unigenes, of which 68,464 were assigned to biological, 78,107 unigenes molecular function and 44,414 to cellular components. Significantly enriched GO terms in DEGs were related to oxidations reduction biological process, protein binding and oxidoreductase activity. This transcriptome data on pomegranate could help in understanding resistance and susceptibility nature of cultivars and further detailed fine mapping and functional validation of identified candidate gene would provide scope for resistance breeding programme in pomegranate.

1. Introduction

Pomegranate (*Punica granatum* L.) is an important fruit crop in countries such as India, Iran, China, and Turkey (Soloklui et al., 2012). It is a perennial fruit tree that is native from Iran to the Himalayan Mountains in northern India (Morton, 1987, Soloklui et al., 2012). India and Iran collectively produce more than half the world's pomegranate (Petersen et al., 2010, Sharma et al., 2010). Pomegranate aril, seed, rind, flower, bark and root produce different types of biologically active compounds and phytochemicals, including gallotannins, ellagic acid, flavonoids, antioxidants,

* Corresponding author.
E-mail address: nripendras72@gmail.com (N.V. Singh).
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terpenoids and alkaloids, and are used in treating diseases such as atherosclerosis, breast cancer, skin cancer, and prostate cancer (Bayazit and Caliskan, 2018, MayuoniKirshinbaum and Porat, 2014, Syed et al., 2013, Ophir et al., 2014). With the characteristics of medicinal properties, sustainability in diversified environment and soil conditions, less irrigation water requirement, high returns on investment and huge export and domestic demand, pomegranate is a good choice for cultivation. Thus, it is an important fruit crop for ensuring the livelihood of farmers in water-scarce regions (Bhandari, 2012). However, during last one and half decades, pomegranate growers in India have been in dire straits because of the severe outbreak of bacterial blight disease caused by *Xanthomonas axonopodis* pv. *punicae*, which has resulted in heavy yield losses (Sharma et al., 2017).

The bacterial blight-causing pathogen in pomegranate, *X. axonopodis* pv. *punicae*, was identified in 1952 (Hingorani and Mehta, 1952) but was not considered a serious threat. Gradually, the disease has become a serious threat and outbreaks have reached an epiphytotic level in several parts of central India, resulting in yield loss both in terms of quality and quantity (Kumar et al., 2011, Sharma et al., 2017, Sharma et al., 2010). Bacterial blight disease of pomegranate has been reported in countries such as Pakistan (Akhtar and Bhatti, 1992), South Africa (Petersen et al., 2010), and Turkey (Icoz et al., 2014).

X. axonopodis pv. *punicae* is a gram-negative rod-shaped and non-sporing bacterium. In general, the mode of infection of this pathogen is *via* rain, irrigation water, infected planting material, insect vectors, farm implements and humans. This pathogen also spreads *via* air and enters the host through natural openings or physical injuries to plants, the daily average temperature between 25 and 35 °C and RH > 30% favours the rapid blight spread. (Sharma et al., 2017). The blight affects all above ground parts of the plant of which the fruits are the most vulnerable (Sharma et al., 2010, Singh et al., 2015). After inoculation, *X. axonopodis* pv. *punicae* infection has three prominent stages in the host. The stage 1 is associated with the appearance of water soaked lesions on leaves and fruit, the stage 2 is associated with necrotic dark brown/blackish brown spots with a yellow halo against light on leaf and necrotic dark brown/blackish depressed lesions on fruit, and stage 3 is characterized by large blighted areas with dried silvery bacterial ooze on leaves and enlarged necrotic lesions with cracks and dried white encrustation of bacterial ooze on fruit (Sharma et al., 2017). PCR based early and reliable diagnostic techniques are available for detection and confirmation of *X. axonopodis* pv. *punicae* infection in pomegranate (Sharma et al., 2017, Doddaraju et al., 2019).

Previously, transcriptome analysis based on two phenotypically different pomegranate cultivars led to the identification of simple sequence repeats (SSRs) and also single nucleotide polymorphisms (SNPs) in pomegranate (Ophir et al., 2014). A few attempts have been made to develop a genetic map of economically important traits in pomegranate based on the transcript markers enriched with quantitative trait loci (Harel-Beja et al., 2015). Whole genome sequencing and limited transcriptome analysis using fruit peel, leaves, roots, flowers along with their parts and fruit are available to add to the large scale comparative transcriptome data generated in the current investigation, all these can result in a tremendous increase in genomics resources on pomegranate (Ono et al., 2011, Ophir et al., 2014, Qin et al., 2017, Yuan et al., 2018).

Although these resources provide good information about the biology of pomegranate, host–pathogen interactions with special reference to *X. axonopodis* pv. *punicae via* RNA-Seq is not available. To the best of our knowledge, this is the first report describing the identification of the differentially expressed candidate genes responsible for resistance against pathogen infection by using NGS technologies such as RNA-Seq analysis. Here, we report results from high-throughput Illumina sequencing, *de novo* assembly and functional annotation of the pomegranate transcriptome under *Xanthomonas* infection and the identification of differentially expressed genes (DEGs) in susceptible and moderately resistant pomegranate genotypes. The transcriptome data generated could help in understanding resistance and susceptibility nature of cultivars and further detailed fine mapping and functional validation of identified candidate gene would provide scope for resistance breeding programme in pomegranate.

2. Material and methods

2.1. Experimental site and climatic conditions

The study was carried out at Kegaon Research Farm, ICAR-National Research Centre on Pomegranate, Solapur, India, having 17°43′ N latitude, 75°50′ E longitude and 486 m altitude from mean sea level. During the experimental period, the minimum average temperature and maximum average temperature of the month ranged from 18.19 °C to 33.16 °C and the mean relative humidity during the period was 66.87% with average wind velocity 6.37 ms^{-1}.

2.2. Plant material and challenge inoculation

'Bhagawa' is India's most commercial but blight susceptible pomegranate cultivar having red rind and sweet soft red arils where as IC 524207 is a wild type pomegranate genotype having small acidic fruits but moderately resistant to blight. These pomegranate plants were inoculated by spraying pure culture of *X. axonopodis* pv. *punicae* under conditions favourable for blight infection (average daily temperature 25-35 °C and RH > 30%). The culture was maintained at the Plant Pathology Laboratory of ICAR-NRCP. The pathogen was isolated from infected samples on NGA medium. Briefly, samples were surface-sterilized in sodium hypochlorite (2%) for 2–3 min, then washed with sterilized deionized water three times and blot dried. The samples were macerated on a sterilized glass slide and streaked on the NGA. The culture plates were incubated at 28 ± 1 °C. The colonies that were developed after 48–72 h of incubation were chosen for further confirmation. The colonies of *X. axonopodis* pv. *punicae* were identified by colour, texture, morphology and characteristic brown pigmented fuscan production. The bacterial identity was further confirmed by gyrB-specific PCR amplification that is known to yield a 491-bp amplicon in *X. axonopodis* pv. *punicae* (Mondal et al., 2012, Sharma et al., 2017). To prepare the inoculum, a loop full of bacterium was inoculated in nutrient glucose broth with constant shaking at 100 rpm. Bacterial suspension was diluted to 10^{-7} with shaking at 100 rpm. The spray inoculation method was used for challenge inoculation under field conditions (Fig. 1B) and symptoms on leaf and fruits were confirmed as bacterial blight infection by visual identification (Fig. 1C–K) and *in vitro* culture characteristics of *X. axonopodis* pv. *punicae* (Sharma et al., 2017).

2.3. Preparation of samples for RNA-Seq analysis

We used 12 samples (pooled total RNA samples from three technical replicates) in this study: three leaves and fruit samples of Bhagawa (pooled sample of three technical replicates) with progressive stages of blight infection symptoms from stage 1 to 3 were collected, LS_1 i.e., first stage of infection on leaf of Bhagawa appeared on 5th day after challenge inoculation (days to post inoculation-dpi), second stage- LS_2 (8 dpi), third stage- LS_3 (11 dpi) and first stage on fruit of 'Bhagawa'-FS_1 (11dpi), second stage-FS_2 (15 dpi), third stage-FS_3 (21 dpi), respectively; alongwith non-inoculated control leaf and fruit samples of Bhagawa

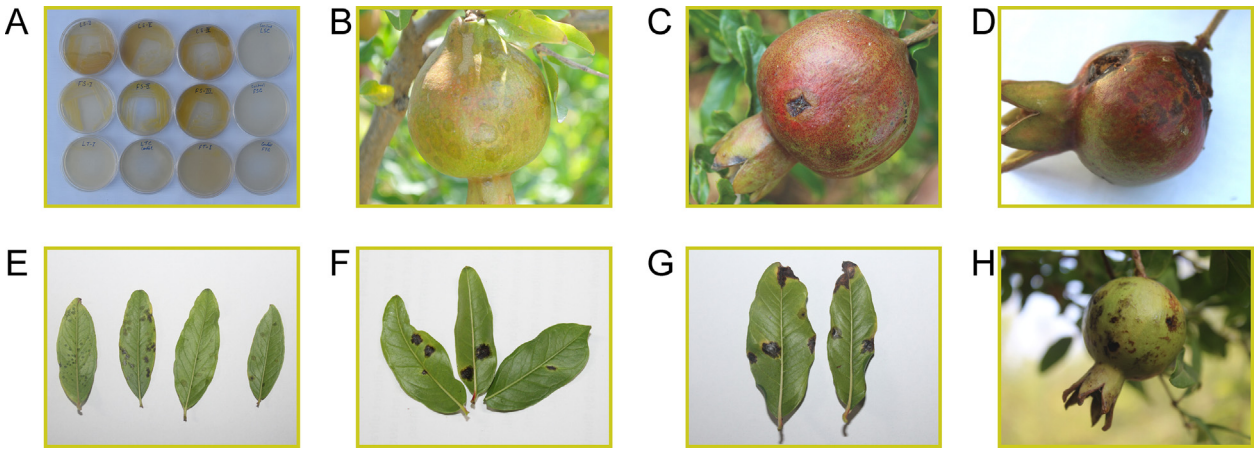

Fig. 1. (A) Stage-wise cultures of *Xanthomonas axonopodis* pv. *punicae*. (B) First stage of *Xanthomonas* infection on fruits of 'Bhagawa' at 11 dpi (susceptible variety) — water-soaked lesions on leaves and appearance of oily patches (C) Second stage of infection at 15 dpi — appearance of necrotic dark brown/blackish brown depressed lesions. (D) Third stage of infection at 21dpi — enlarged necrotic lesions with cracks and dried white encrustation of bacterial ooze on lesions. (E) First stage of infection on leaf of Bhagawa at 5 dpi (F) Second stage on Bhagawa leaf at 8 dpi— necrotic dark brown/blackish brown spots with yellow halo against light. (G) Third stage on Bhagawa leaf at 11 dpi — large blighted areas with dried silvery bacterial ooze. (H) First stage of infection on fruit of IC524207 at 14 dpi.

(LS_C, FS_C); three pooled samples each of leaf and fruit of IC 524204 at stage 1 of blight infection was taken (LT_1: 7 dpi and FT_1: 14 dpi) along with control samples of IC 524204 (LT_C, FT_C). The samples of 2nd and 3rd stages of infection on both leaf and fruit of IC 524204 were ignored as symptom progression was very slow and overlapping infection symptoms were observed at stage 1 and 2, however infection stage 3 (cracking of fruits) was not observed on fruits of tolerant genotype. Total RNA was extracted from all 12 samples by using the Nucleospin RNA isolation kit. RNA quantity and quality were determined by using Qubit Flurometer 3.0 with absorbance ratios at 260 nm/280 nm by using Nanodrop. The RNA integrity was tested by separating an aliquot of the RNA sample on 1% agarose gel electrophoresis. Quality was assessed by visualizing and checking the appearance of ribosomal RNA bands and by lack of degradation products. The integrity of RNA was re-confirmed by using Bioanalyzer 2100 (Agilent, Folsom, CA). Total RNA from three technical replicates were pooled for each sample and samples were enriched for mRNA by using oligo (dT) beads followed by removal of rRNA by using a Ribo-Zero kit. Then, mRNA was fragmented randomly into small pieces by adding the fragmentation buffer provided with the Illumina mRNA-seq kit (Illumina, San Diego, CA). The fragmented mRNA was reverse-transcribed by using random hexamers and further amplified to produce double-stranded DNA. Repair of the fragment ends and 3′-end adenylation were implemented with the NEBNext™ DNA Sample Prep Reagent Set 1 (New England BioLabs, Ipswich, MA). The paired end adaptors were linked to the ends of the DNA fragments. The resulting cDNA templates were gel-purified and PCR-enriched. Quality of the mRNA-Seq library was verified by using Bioanalyzer.

2.4. Illumina sequencing, quality control and transcriptome de novo assembly

The quantified mRNA-Seq libraries were sequenced by using Illumina HiSeq 2500. We used DrSeq, an automated RNA Seq pipeline developed by Nucleome Informatics, for data analysis. FastQC was used to check the quality of the raw data and to avoid low-quality bases that result in mis-assemblies at the time of assembly. FastQC checks the quality of the data by considering sequencing error rate (e) and sequencing base quality (Qphred) value or quality score (QC). The filtered high-quality reads were used for downstream analyses. The clean transcriptome reads were assembled *de novo* by using Trinity software (Grabherr et al., 2011).

2.5. Functional annotation and classification of genes

Unigenes were searched against seven public databases: the NCBI non-redundant protein sequence database (Nr database) (https://www.ncbi.nlm.nih.gov/refseq/about/nonredundantproteins/); the NCBI nucleotide sequence database (Nt database) (https://www.ncbi.nlm.nih.gov/nucleotide?cmd=search); the KO database (https://www.genome.jp/kegg/ko.html); SwissProt (Boeckmann et al., 2003); Proteinfamily (Pfam) database (Bateman et al., 2004); Geneontology (GO) database (Consortium, 2004) and Eukaryotic orthologous groups (KOG) database by using BLASTX. The BLASTX results were imported to Blast2GO v2.5 (Conesa and Götz, 2008) for functional classification. We used in house scripts for mapping, retrieving and allocation of GO terms to genes. These genes were also annotated with unique enzyme codes (ECs) and KEGG maps (http://www.genome.jp/kegg) by using KAAS software (Kanehisa and Goto, 2000, Kanehisa et al., 2011). The genes present in the transcriptome of bacterial blight-challenged tissues were classified into three GO categories: biological process, cellular component and molecular function.

2.6. Estimation of transcript abundance and analysis of differential expression

The quantification of transcripts involved RNA-Seq with Expectation Maximization RSEM 1.2.7 (https://www.encodeproject.org/software/rsem/). To estimate the abundance of each samples at various stages, the expression-normalizing fragments per kilobase of exon fragments per million mapped (FPKM) were calculated by using RSEM 1.2.7. P-values for differential expression of genes among two samples was calculated by using the likelihood ratio test in the R package, DEGseq (Wang et al., 2009; Marioni et al., 2008). The P-values were adjusted by using the Q-value (Storey and Tibshirani, 2003). Q-value < 0.05 was used to identify the significantly differentially expressed genes. Finally, the combination of Q-value (<0.05 and fold change (>2 and < -2) was used to identify up- and down regulated genes.

2.7. Prediction of R genes among the upregulated genes of different sample combinations

We used all upregulated gene IDs for all combinations of samples as described earlier. We downloaded the *R* gene protein sequences of different plant species from (http://prgdb.crg.eu/

wiki/Download) and a local database was constructed. By using the protein sequences obtained by translating the upregulated unigenes of the combinations of the samples compared, BLASTP search was performed between the protein sequences of upregulated genes of all combinations and the local database of *R* proteins.

2.8. Total RNA isolation and first-strand cDNA synthesis

Total RNA was isolated from pomegranate leaf and fruit tissues of the 12 samples of Bhagawa and IC 524204 (with three technical replicates) by using the NucleoSpin RNA plant kit (Macherey–Nagel, Duren, Germany) according to the manufacturer's protocol with slight modifications. The RNA concentration and purity were assessed by using the Qubit 3.0 fluorometer. The integrity was checked on 1% (w/v) agarose gel. Total RNA samples (800 ng) each with 260/280 ratio 2.0 to 2.1 and 260/230 ratio 2.1 to 2.3 were used for cDNA synthesis for all samples. Total RNA from 3 technical replicates of each sample were pooled together. First-strand cDNA was synthesized by using a High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, USA), following the manufacturer's instructions, in a final volume of 20 μl. The final cDNA samples were diluted three-fold and used as a template in RT-qPCR.

2.9. Quantitative real-time PCR (RT-qPCR)

The RT-qPCR reactions were performed with a Light Cycler 96 system (Applied Biosystems StepOne Plus, USA) with the Hi-SYBr Master Mix including Taq polymerase (HiMEDIA, India) in 96-well optical reaction Roche plates. Each reaction was performed in triplicate with 5 μl SYBR Green Master, 1 μl template cDNA, 0.2 μl each primer (10 μM), and 3.6 μl RNase-free water with a total volume of 10 μl. The RT-qPCR profile was 95 °C (2 min), 40 cycles of 95 °C (5 s), 60 °C (30 s) with fluorescent signal recording and 72 °C for 30 s. The melting curve was obtained by using a high-resolution melting profile performed after the last PCR cycle, 95 °C for 15 s followed by a constant increase in the temperature from 65 °C (15 s) to 95 °C (1 s). All samples in three technical replicates were repeated to confirm results.

3. Results

3.1. De novo assembly and clustering of transcriptome from infected tissues of pomegranate

The pomegranate variety Bhagawa is cultivated type and highly susceptible to *X. axonopodis* pv. *punicae* infection and the genotype IC 524207 wild type collected from hills of Himalayas, is moderately resistant to *X. axonopodis* pv. *punicae* infection. With a high-throughput IlluminaHiSeq 2500 sequencing platform, we obtained 50,10,95,768 raw transcriptome reads from both susceptible Bhagawa and moderately-resistant IC 524207 from *X. axonopodis* pv. *punicae* infected and control fruit and leaf samples, which accounted for approximately 75.16 GB of sequenced data (NCBI Accession No. PRJNA361285). Furthermore, raw reads were subjected to quality control (Fast QC) to obtain clean transcriptome reads of 48,58,02,868, which represented about 72.87 GB (96.95%) of high-quality sequence data with a >92% quality check score for all samples and 94% for most samples at Q30 with high GC content (>47.50). The data on sequencing, quality of data and error rates are in Table 1. All these filtered reads were used in transcriptome assembly. The minimum, mean, median, and max length as well as N50 and N90 value and total nucleotides sequenced were 201, 1184, 642, 23878, 2081, 471, and 22,29,23,628, respectively (Table 2). The transcriptome assembly in our study resulted from 188,337 unigenes with maximum and minimum read lengths of 23,878 and 200 bp, respectively, with an average size of assembled unigene of 1184 bp and 34,626 unigenes >2 kb in size (Table 3, Fig. 2A and B), which indicated increased coverage as well asthe depth and high quality assembly of the sequenced transcriptome data.

3.2. Similarity searches of pomegranate transcriptome

Functional annotation of unigenes was performed by using similarity searches. About 1,60,683 of 1,88,337 transcripts (85.31%) were annotated in at least in one of the selected databases. Furthermore, about 21,606 transcripts (11.47%) were annotated in all seven databases (Table 4) (Fig. 3A). The individual count of total number of genes annotated against the selected databases

Table 1
RNA Seq data. LS_1, LS_2, LS_3, LS_C represent the leaf sample and control sample of Bhagawa infected with *Xanthomonas axonopodis*pv. *punicae*, along with stage of infection. FS_1, FS_2, FS_3 represent the infected fruit sample and control sample of Bhagawa along with stage of infection. LT_1, LT_C represents infected leaf sample of IC 524207 with first stage of infection along with its control sample. FT_1, FT_C represent the infected fruit sample at first stage of infection and its control sample of IC 524207.

Sample	Raw Reads	Clean Reads	Clean Bases Size (GB)	Error %	Q20%	Q30%	GC Content
LS_1	58,279,956	56,911,978	8.54	0.01	98.21	95.57	50.07
LS_2	64,644,180	63,438,302	9.52	0.01	98.23	95.59	49.32
LS_3	57,946,290	56,346,098	8.45	0.01	97.77	94.31	49.85
FS_1	20,630,104	19,691,646	2.95	0.01	97.35	93.47	54.63
FS_2	19,578,544	18,764,108	2.81	0.02	97.06	92.7	54.39
FS_3	21,913,568	21,063,454	3.16	0.02	97.01	92.57	54.29
LS_C	49,579,848	48,131,138	7.22	0.02	96.83	92.01	50.84
FS_C	49,733,888	47,679,996	7.15	0.01	97.94	94.85	49.9
LT_1	59,916,394	58,049,980	8.71	0.01	97.8	94.69	49.99
FT_1	23,247,992	22,516,058	3.38	0.01	97.56	93.84	47.5
LT_C	55,469,742	53,602,820	8.04	0.01	97.91	94.53	51.03
FT_C	20,155,262	19,607,290	2.94	0.01	97.83	94.21	48.23

Table 2
Data for transcripts. Minimum length represents the minimum length of the transcript. Mean length represents the average length of transcript. Maximum length represents the transcript length with maximum base pairs.

Minimum length of Transcript	Mean Length of Transcript	Median Length of Transcript	Maximum Length of Transcript	N50 value	N90 value	Total nucleotides
201	1184	642	23,878	2081	471	222,923,628

is shown in Table 4. BLASTX searches showed that about 51.6% of annotated sequences belong to other species (many different species of plants mixed together), followed by 29.3% in *Eucalyptus grandis* (Fig. 3B), which shows the evolutionary relationship of pomegranate with *Eucalyptus*. However, very small proportions of sequences were annotated with reference to *Vitis* (5.8%), *Theobroma cacao* (5.2%), *Ricinus commuis* (3.9%), *Jatropha curcas* (3.2%) and *Gossypium raimndii* (2.6%) (Fig. 3B).

Table 3
De novo transcript assembly and its length distribution. Unigene interval length represents the length of the unigenes.

Unigene interval length	200–500 bp	500–1 Kbp	1–2 kbp	>2 kbp	Maximum length	Total
Total no. of unigenes	71,157	49,611	32,943	34,626	23,878	188,337

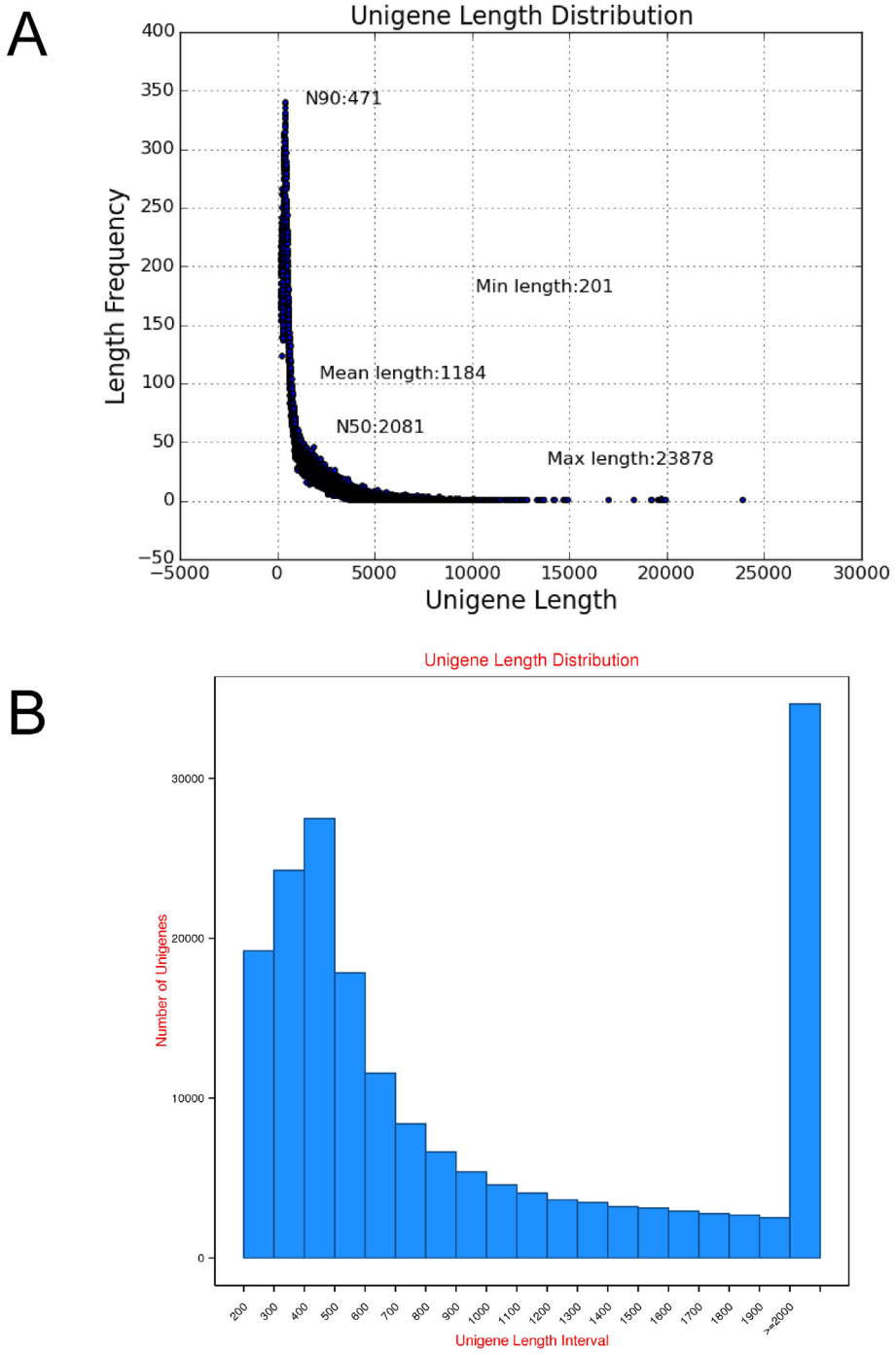

Fig. 2. (A) Distribution of unigenes based on length frequency. (B) Unigenes length distribution and their number.

3.3. Functional classification of pomegranate unigenes based on GO, KEGG and KOG

Pomegranate unigeneswith non-redundant annotations were functionally annotated by Gene Ontology (GO) analysis for biological process, molecular function and cellular component. From a total of 90,485 pomegranate unigenes, 68,464 were assigned to biological functions. In total, 78,107 unigenes were assigned to molecular function and 44,414 to cellular components (Fig. 4A). Functional classification of transcripts with respect to Kyoto Encyclopedia of Genes and Genomes (KEGG) showed most were under the metabolism category and gene processing category (Fig. 4B). The transcripts we obtained were aligned to the Eukaryotic orthologous groups (KOG) database to predict and classify their possible functions. Transcripts falling under categories "post translational modification", "protein turnover", and "chaperones" were highly clustered (Fig. 4C).

3.4. Analysis of differentially expressed genes

In order to compare gene expression levels under different experimental conditions, an FPKM frequency distribution graph and box plot analysis were performed. Both these graphs display overall gene expression levels in test samples. The significant differences for mean gene expression levels with wide range between FT_1 and FT_C was noticed in comparison to all other conditions as reflected from both the FPKM density distribution and box plot graphs (Fig. 5A and B). It was also interesting to note that fruit tissues of tolerant genotypes displayed large number of genes expressed at stage 1 compared to susceptible plant tissues at different stages. Further, FT_1 has higher expression compared to control FT_C indicating induced activation of defense related genes after infection at stage 1. In-order to find genes with similar expression patterns under various experimental conditions hierarchical FPKM clustering analysis was performed using the log10 (FPKM + 1) values. By clustering genes with similar expression pattern, it is possible to discern unknown functions of previously characterized genes or the functions of unknown genes. Genes within the same cluster exhibit the same trends in expression under different conditions. Heatmap showed many genes which were up or down regulated among different conditions. We found higher differential up and down regulation of genes in fruit tissues FT_1 and FT_C. However, higher differential down regulation of genes in FS_1, 2 and 3 as compared to FS_C (Fig. 5C).

Table 4
Number of annotated reads in different databases and their percentage annotation in each database considered in the analysis. About 85.1% of unigenes were annotated in at least one database, with about 11.7% of genes annotated in all databases.

Database used for annotation	Number of annotated reads	Percentage of annotation
NR	94,535	50.19
NT	129,632	68.82
KO	39,558	21
SwissProt	124,999	66.36
PFAM	89,152	47.33
GO	90,485	48.04
KOG	54,102	28.72
Annotated in all databases	21,606	11.47
Annotated in at least one database	160,683	85.31
Total genes	**188,337**	

3.5. Identification of DEGs between control and infected fruit and leaf samples of IC 524207 and Bhagawa

To understand the transcriptional response of the host at the time of infection, we identified the DEGs between control and infected samples. We selected the combinations of control and infected samples shown in Table 5. In our analysis, we ignored infection stage 2nd and 3rd of leaf and fruits in IC 524507 as symptom progression was very slow and overlapping of infection symptoms was observed at stage 1 and 2, however infection stage 3 was not observed on fruits and leaves of tolerant genotype.

3.6. Identification of DEGs in leaf and fruit samples of control and infected tissues of IC 524207

We identified DEGs between the control and infected fruit samples (FT_1vs FT_C) of IC 524207 at stage 1 (14 dpi). In infected fruit samples (FT_1 vs FT_C), about 937 and 1047 genes were up- and downregulated, respectively (Table 6) (Supplementary dataset 1 & 2). Similarly, a total of 622 and 593genes were up- and downregulated in infected leaf samples versus the control (LT_1 vs LT_C) at 7 dpi (Table 6) (Supplementary dataset 3 & 4).

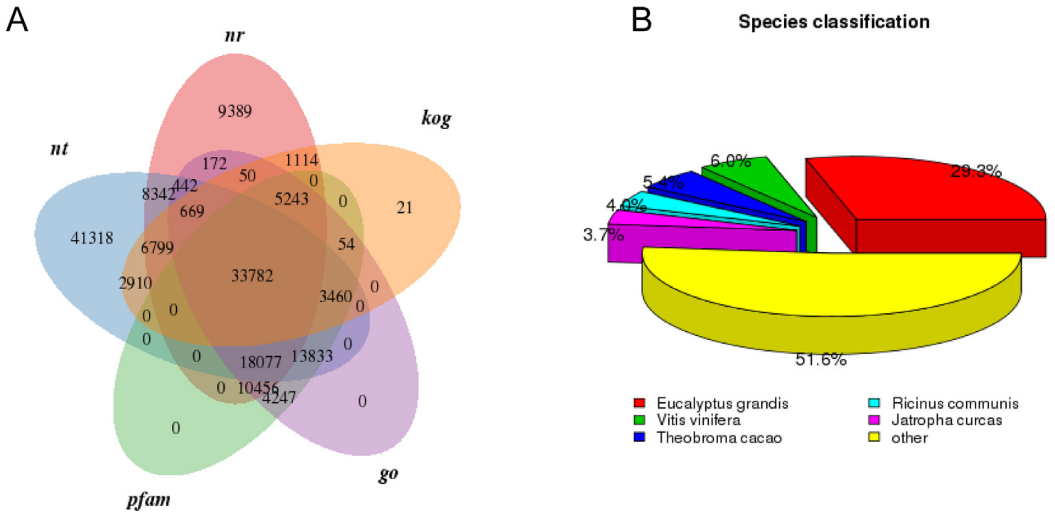

Fig. 3. (A) Venn diagram of annotation of unigenes in different databases. (B) Homologs of unigenes in other plant species. Pie chart shows that most unigenes have homologs in *Eucalyptus grandis*.

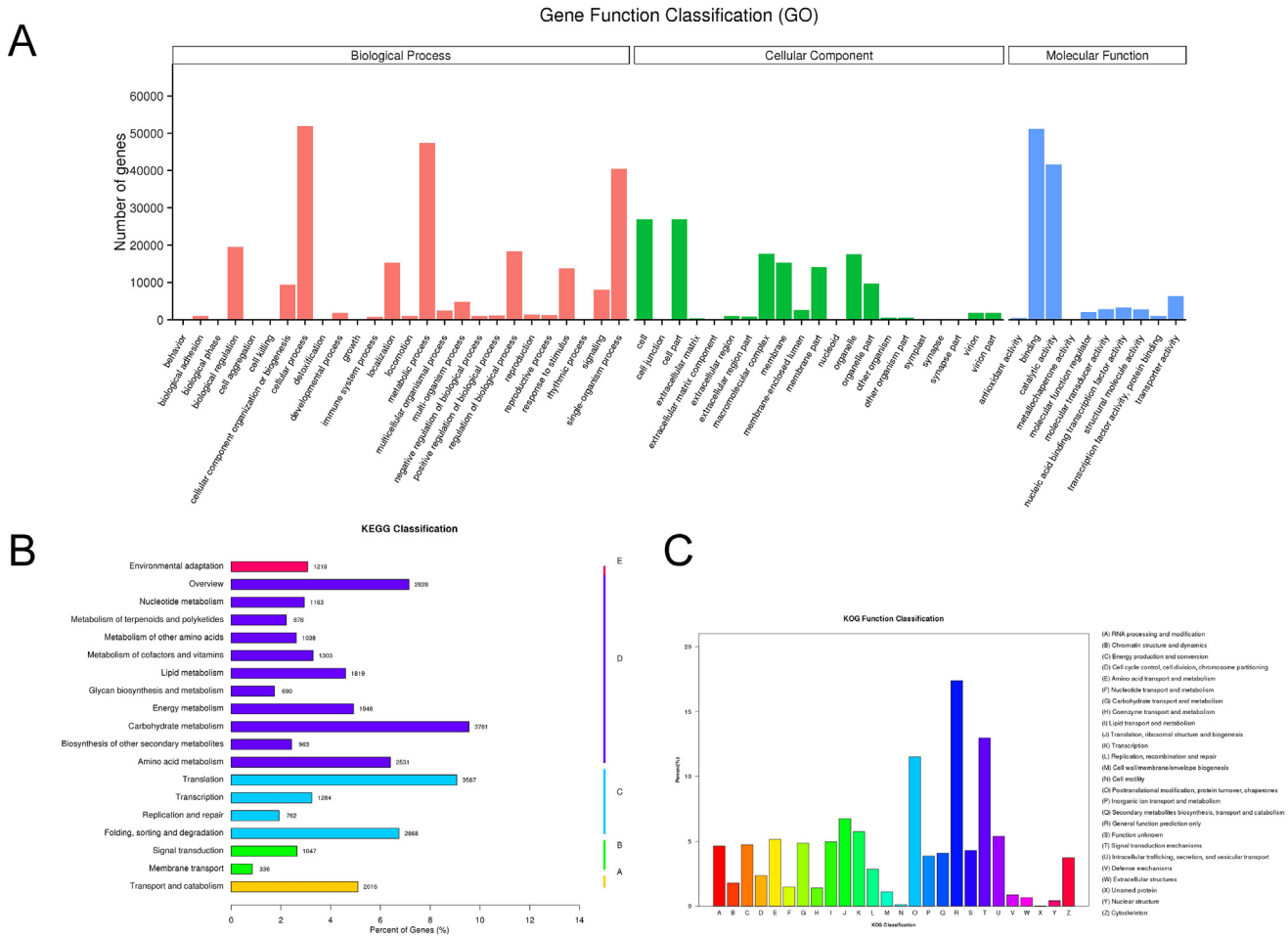

Fig. 4. (A) Bar graph showing the distribution of unigene annotations in GO database. (B) Bar graph showing the distribution of unigene annotations in KEGG database. Most of the genes fall under the carbohydrate metabolism pathway. (B) Bar graph showing the annotations of unigenes in KOG database. Most of the genes fall under general functions.

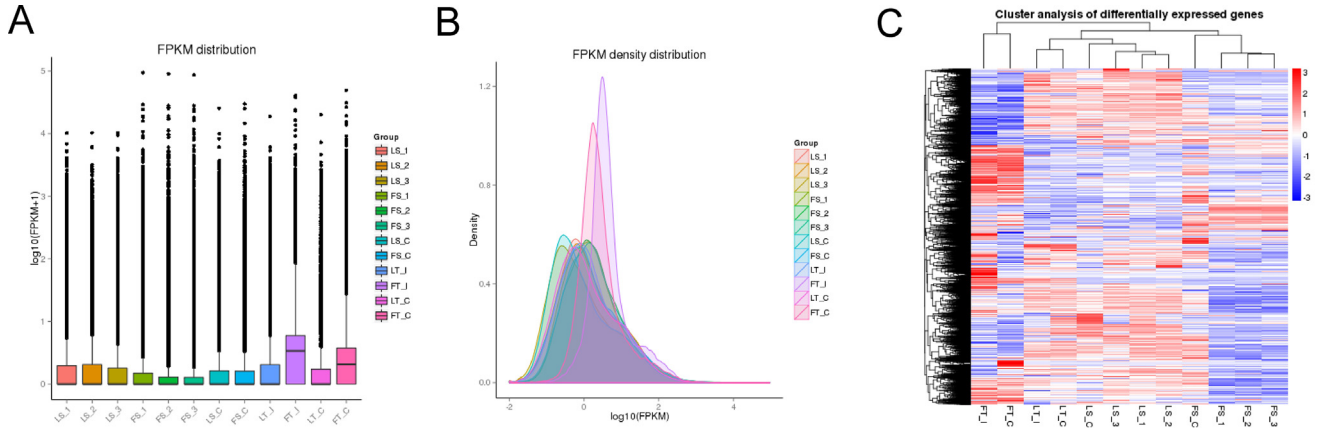

Fig. 5. (A) FPKM density distribution. (B) FPKM box plot. (C) H_cluster heat map for FPKM cluster analysis of differentially expressed genes. Red denotes genes with high expression levels and blue denotes genes with low expression levels. The color range from red to blue represents the log10 (FPKM + 1) value from large to small.

3.7. Identification of DEGs between leaf and fruit samples of control and infected Bhagawa tissues

In the first stage of infection of fruit in Bhagawa, 717 and 2735 genes were up- and downregulated, respectively at 11 dpi (FS_1 vs FS_C) (Table 6) (Supplementary dataset 5 & 6). In leaf infected samples, a total of 595 and 705 genes were up- and downregulated at 5 dpi (Table 6) (Supplementary dataset 7 & 8). In the second stage of infection of Bhagawa fruit, about 779 genes were upregulated and 2740 downregulated with respect to the control at 15 dpi (Table 6) (Supplementary dataset 9 & 10). Similarly, a total of 1101 and 1185 genes were up- and downregulated in the infected samples of leaf with respect to the control at 8 dpi (Table 6) (Supplementary dataset 11 & 12). In the third stage of pathogen infection, 795

and 2656 genes were up- and downregulated in infected fruit samples at 21 dpi (Table 6) (Supplementary dataset 13 & 14) and 952 and 1143were up- and downregulated in infected leaf samples with respect to the control at 11 dpi (Table 6) (Supplementary dataset 15 & 16)

Table 5
Combinations of the samples considered for understanding the differential expression of the genes between the control samples and infected samples. FT and LT represent the infected fruit and leaf samples of IC 524207, respectively. FS and LS represent the infected fruit and leaf samples of Bhagawa, respectively. C represents the control.

Days to post inoculation	Combination
14 dpi	FT_1 and FT_C
7 dpi	LT_1 and LT_C
11 dpi	FS_1 and FS_C
15 dpi	FS_2 and FS_C
21 dpi	FS_3 and FS_C
5 dpi	LS_1 and LS_C
8 dpi	LS_2 and LS_C
11 dpi	LS_3 and LS_C

Table 6
Combination of samples compared and total number of up- and downregulated genes in infected fruit and leaf samples of IC 524207 and Bhagawa pomegranate. FT and LT represent the infected fruit and leaf samples of IC 524207. FS and LS represent the infected fruit and leaf sample of Bhagawa. C represents the control.

Sample No.	Stage of infection	Combination of sample	Total no. of upregulated genes	Total no. of downregulated genes
1	First stage	FT_1 and FT_C	937	1047
2	First stage	LT_1 and LT_C	622	593
3	First stage	FS_1 and FS_C	717	2735
4	First stage	LS_1 and LS_C	595	705
5	Second stage	FS_2 and FS_C	779	2740
6	Second stage	LS_2 and LS_C	1101	1185
7	Third stage	FS_3 and FS_C	795	2656
8	Third stage	LS_3 and LS_C	952	1143

Table 7
Combinations of samples considered for understanding the differential expression of the genes at each stage. IC 524207 represents the moderately resistant pomegranate variety, L represents leaf sample, F represents fruit sample, S represents susceptible and T represents tolerant.

Sample No.	Combination
1	FS_1 and FT_1
2	LS_1 and LT_1
3	FS_2 and FT_1
4	FS_3 and FT_1
5	LS_2 and LT_1
6	LS_3 and LT_1

3.8. Comparative study of the identification of up and down-regulated genes among infected samples of IC 524207 and Bhagawa

We compared infected fruit and leaf samples at different stages (dpi) in IC 524207 and Bhagawa along with control to retrieve the DEGs. The combinations for this analysis are shown in Table 7. Table 8 represents the comprehensive information for the compared samples and total number of DEGs at each stage of infection. At the first stage of fruit infection in Bhagawa and IC 524207, 2102 and 4221 genes were up- and downregulated, respectively. In comparing the second stage of fruit of Bhagawa and the first stage of IC 524207, 2305 and 4447 genes were up-and downregulated. Similarly, in comparing the Bhagawa third stage and IC 524207 first stage for fruit, 2347 and 4455 genes were up- and downregulated, respectively. In comparing infected leaf samples at the first stage of Bhagawa and stage 1 of IC 524207, 1011 and 1077 genes were up- and downregulated, respectively. Furthermore, 771 and 768 genes were up- and downregulated in leaf samples at stage 2 of Bhagawa and stage 1 of IC 524207. Moreover, 1302 and 1240 genes were up- and downregulated in comparing stage 3 of Bhagawa and stage 1 of IC 524207.

3.9. Identification of up and down-regulated genes among infected leaf and fruits samples of Bhagawa.

While comparing leaf and fruit tissues of different progressive blight infection stages, the maximum number of differentially expressed genes were found in FS_1 vs FS_3 (399 downregulated and 358 upregulated) (Table 9 and 10). However, GO enrichment was higher in LS_1 vs LS_3. The most significantly enriched GO term were related to catalytic activity and metabolic process (Fig. 6). Major KEGG enriched pathways for DEGs were related to ribosome, photosynthesis, plant pathogen interaction, phenylpropanoid biosynthesis and plant hormone signal transduction.

3.10. Identification of R genes among the upregulated genes of all combinations

We identified the *R* genes among upregulated genes of different combinations (Table 11). The comparison of FS_C vs FT_C and FS_2 vs FT_1 had more numbers (about 175 and 140, respectively) of *R* genes.

Table 9
Identification of up and down-regulated genes among infected leaf and fruits samples of Bhagawa.

Sample No.	Sample Details	Upregulated genes	Downregulated genes
1	FS_1 vs FS_2	288	374
2	FS_1 vs FS_3	399	358
3	FS_2 vs FS_3	355	295
4	LS_1 vs LS_2	296	269
5	LS_1 vs LS_3	376	265
6	LS_2 vs LS_3	330	223

Table 8
Combination of the samples compared and total number of up -and downregulated genes in infected fruit and leaf samples of IC 524207 and Bhagawa pomegranate between each stage of infection.

Sample No.	Sample combination	Stage of infection of IC 524207	Stage of infection of Bhagawa	Total upregulated genes	Total downregulated genes
1	FS_1 and FT_1	First Stage	First Stage	2102	4221
2	LS_1 and LT_1	First Stage	First Stage	1011	1077
3	FS_2 and FT_1	First Stage	Second Stage	2305	4447
4	LS_2 and LT_1	First Stage	Second Stage	771	768
5	FS_3 and FT_1	First Stage	Third Stage	2347	4455
6	LS_3 and LT_1	First Stage	Third Stage	1302	1240

Table 10
Gene Ontology classification of significantly enriched up- and down-regulated genes among the different diseased leaf and fruit samples of 'Bhagawa'

Samples compared	Gene Ontology classification of upregulated genes			Gene Ontology classification of major downregulated genes		
	Biological Processes	Cellular Processes	Molecular Functions	Biological Processes	Cellular Processes	Molecular Functions
FS_1 vs FS_2	73	43	83	85	45	98
FS_1 vs FS_3	68	37	76	96	49	108
FS_2 vs FS_3	67	33	75	75	38	84
LS_1 vs LS_2	122	71	129	145	69	147
LS_1 vs LS_3	126	61	132	178	89	188
LS_2 vs LS_3	103	50	114	125	81	143

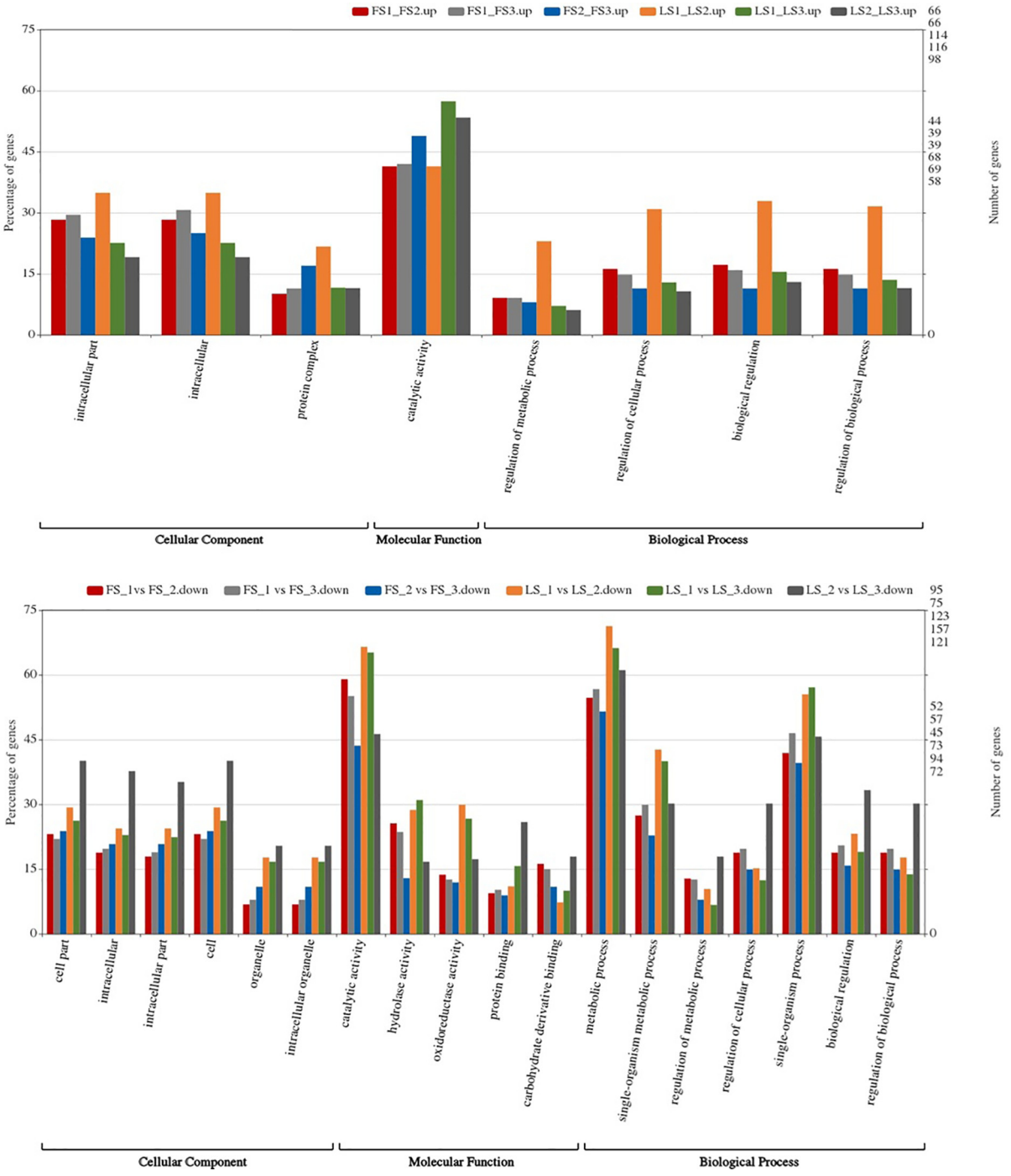

Fig. 6. GO enrichment of up and downregulated genes of infected leaf and fruit transcriptome of Bhagawa.

Table 11
The predicted upregulated *R* genes in each combination. Among the compared samples, FS_C vs FT_C had the highest number of *R* genes among the upregulated genes.

Sample Combination	Total number of upregulated gene matching *R* genes
FS_1 vs FS_C	72
FS_1 vs FT_1	115
FS_2 vs FS_C	70
FS_2 vs FT_1	140
FS_3 vs FS_C	72
FS_3 vs FT_1	126
FS_C vs FT_C	175
FT_1 vs FT_C	83
LS_1 vs LS_C	54
LS_1 vs LT_1	93
LS_2 vs LS_C	114
LS_2 vs LT_1	76
LS_3 vs LS_C	75
LS_3 vs LT_1	110
LS_C vs LT_C	73
LT_1 vs LT_C	49

3.11. Experimental validation of DEGs

To validate the results of RNA-Seq, we used RT-qPCR to validate the expression of three genes coding for xyloglucan endotransglycosylase, superoxide dismutase, and alcohol dehydrogenase. We selected these genes randomly from the data generated from DEG-seq analysis. We designed the forward and reverse primers for the genes and amplified the genes in all 12 samples (Table 12). The gene that codes for xyloglucan endotransglycosylase was slightly over-expressed in infected leaf samples of Bhagawa at stage 1 and 3 of infection as compared with the control (Fig. 7A). Similarly, the gene that codes for xyloglucan endotransglycosylase was under-expressed in infected leaf samples of IC 524207 at stage 1 of infection versus the control. Xyloglucan endotransglycosylase showed higher expression in infected fruit samples of Bhagawa at stage 2 of infection versus the control. We did not obtain any data for infected fruit samples of Bhagawa at stage 1 and stage 3 or IC 524207 at stage 2 (Fig. 7A).

For the gene coding for alcohol dehydrogenase, the control sample of infected leaf of Bhagawa had higher expression than at any of the stages (Fig. 7B). We did not obtain any data for the control infected leaf samples of IC 425207. Similarly, we did not obtain any data from infected fruit samples of Bhagawa at stage 1 and 2 (11 and 15 dpi), but the expression of alcohol dehydrogenase was similar at stage 3 of Bhagawa and its control in infected fruit samples.

For the gene coding for superoxide dismutase, we did not obtain any data for infected fruit samples of Bhagawa at 11 dpi and 21 dpi, but we found over-expression of the gene in infected fruit samples

Table 12
List of forward primer and reverse primers used for RT-qPCR of the selected genes.

S.No.	Name of the gene	Forward Primer	Reverse Primer
1	Xyloglucan endo- transglycosylase	CCTCCCACAGGGTAGAGTAG	GAAAGGGTGACAGAGAACAGAG
2	Alcohol dehydrogenase	GTGGTTTGTAGTTCCCGAAGA	AAGAGGTGATTGCCGAGATG
3	Superoxide dismutase	AATTCAGCCGTACACAGACC	CACTGGACCAAACTCCATCATA

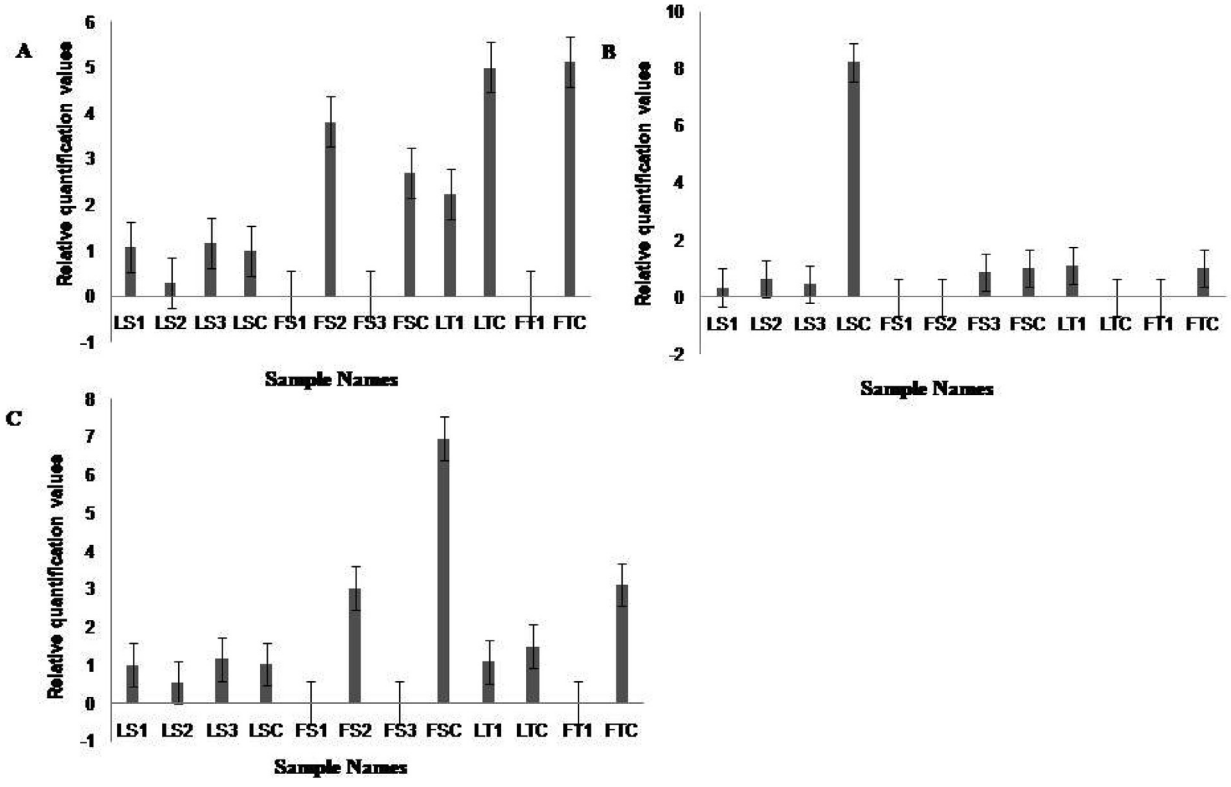

Fig. 7. (A) Bar graph representing the Relative Quantification of the gene coding for Xyloglucan endotransglycosylase. (B) Bar graph representing the Relative Quantification of Superoxide dismutase and (C) Represents the Relative Quantification of Alcohol dehydrogenase. For all the figures on X-axis name of the sample is given and on Y-axis the sample's relative quantification is denoted. In the figures A, B and C few columns are missing where the RT PCR does not show any amplification.

of Bhagawa at stage 2 versus the control (15 dpi). However, we did not obtain any data for IC 524207 at stage 1 of fruit infection (Fig. 7C).

4. Discussion

In 1952, *X. axonopodis* pv. *punicae* was identified in India and was found to be highly evolved and a host-specific pathogen (Hingorani and Mehta, 1952). At the time of infection, the infected leaves show early water-soaked lesions that later convert to necrotic blighting. The infected fruits show coalesced water-soaked lesions leading to necrosis and development of small cracks (Petersen et al., 2010). As an immunological response towards infection, the plant infected with the pathogen at different stages of infection is expected to have a definite transcriptional response (Song et al., 2019). Disease progression and appearance of symptoms on leaf and fruit tissues of tolerant type was slow which confirm moderate resistance in IC 524207 against *Xap* and similar disease reaction pattern was obtained in resistant and susceptible kiwifruit genotypes upon *Pseudomonas syringae* infection in kiwifruit by Song, et al. (2019). Using RNA-Seq, we analyzed the transcriptome of these pomegranate samples infected with *X. axonopodis* pv. *punicae* at a large scale. To the best of our knowledge, these data are the first with high quality in pomegranate as compared with previous reports (Ophir et al., 2014). From the preliminary analysis, before considering the comparative analysis, we found a change in signal transduction genes during pathogenesis (Fig. 4A). Similar observations were found in soybean during *Peronospora manshurica* infection (Dong et al., 2018), which may be due to stronger signals as a result of bacterial invasion. Also, Fig. 4B shows high expression of genes involved in chaperone activities, which are required for degradation of misfolded proteins during bacterial stress and also to increase other protein turnover to cope with the stress condition.

After preliminary analysis, in-depth data analysis was performed in three different ways: comparing the transcriptome data of the infected fruit and leaf samples with the controls, by comparison of progressive disease transcriptome data of Bhagawa leaf and fruits and a comparative study among the infected samples of IC 524207 and Bhagawa.

GO terms obtained by the comparison of the IC 524207 infected fruit samples with their controls (FT_1 vs FT_C) showed that the genes involved in single organism transport and single organism localization biological processes were upregulated and those involved in cell, cell part, intracellular processes were downregulated with infection (Table 13) (Fig. 8A, B).

Comparison of infected leaf samples of IC 524207 and its control (LT_1 vs LT_C) showed upregulation of genes involved in biological process including oxidation-reduction and molecular functions such as oxidoreductase (Table 13) (Fig. 9A, B). At the time of infection, there may be a biotic stimulus to cope with the oxidative stress caused by pathogen infection, so genes involved in oxidation-reduction were upregulated (Li et al., 2016, Liu et al., 2017). We found no significant KEGG pathways in both comparisons.

The comparison of infected fruit samples of Bhagawa and its control (FS_1 vs FS_C)(FS_2 vs FS_C)(FS_3 vs FS_C) shows upregulation of genes involved in oxidation and reduction processes, which predominantly represents the biological process. Under the molecular functions category, oxidoreductase was upregulated, which may be due to the oxidative stress described earlier (Table 13). Under the downregulated category, we did not find significant GO-enriched terms except in FS_1 vs FS_C and FS_2 and FS_C, which showed downregulated genes in the binding and protein binding category. KEGG annotations in all the comparisons showed that metabolic processes such as photosynthesis, oxidative

Table 13
Gene Ontology classification of most significantly enriched up- and down-regulated genes among the different samples compared. NA represents the status of regulation which is not significant as compared with the other processes of GO. The number in the brackets represents the number of genes in that process.

Samples compared	Gene Ontology classification of most significantly enriched upregulated genes			Gene Ontology classification of most significantly enriched downregulated genes		
	Biological Processes and No. of genes involved	Cellular Processes and No. of genes involved	Molecular Functions and No. of genes involved	Biological Processes and No. of genes involved	Cellular Processes and No. of genes involved	Molecular Functions and No. of genes involved
LT_1 vs LT_C	Single organism metabolic process (187) and Oxidation and Reduction (107)	NA	Oxido-reductase (103)	Metabolic processes (315)	NA	Catalytic activity (310) and hydrolase activity (138)
FT_1 vs FT_C	Single organism transport (149) and single organism localization (150)	NA	NA	NA	Cell (318), cell part (318), and intracellular (230)	NA
FS_1 vs FS_C	Single organism Metabolic processes (197) and oxidation–reduction process (110)	NA	Oxidoreductase (95)	NA	NA	Binding (1541)
FS_2 vs FS_C	Single organism Metabolic processes (235) and oxidation and reduction (132)	NA	Oxidoreductase (124)	NA	NA	Binding (1563)/protein binding (753)
FS_3 vs FS_C	Single organism metabolic processes (226) and Oxidation and reduction (130)	NA	Oxidoreductase (121)	NA	NA	NA
LS_1 vs LS_C	Oxidation reduction processes (102)	NA	Oxidoreductase (105), metal ion binding (116), cation binding (117)	Oxidation reduction processes (92)	NA	Oxidoreductase activity (94), Catalytic activity (344)
LS_2 vs LS_C	Metabolic processes (577)		Catalytic activity (519) and ion binding (362)	NA	NA	Catalytic activity (581)
LS_3 vs LS_C	Metabolic processes (490)	NA	Catalytic activity (457)	NA	NA	Catalytic activity (588)

phosphorylation, and protein processing in endoplasmic reticulum were predominantly enriched and most of the upregulated genes were under these categories (Table 14). In general, processes such as photosynthesis in plants were downregulated, which is a defense strategy adopted by the infected plant (Rojas et al., 2014). However, in citrus, during infection with Candidatus *liberibacter* asiaticus, which results in haunglongbing or citrus green disease, (Martinelli et al., 2012) transcriptome analysis showed upregulation of photosynthesis genes. Similar to citrus, when comparing infected fruit samples of Bhagawa to controls, photosynthesis genes were upregulated. A similar consideration was made in analyzing citrus green disease (Martinelli et al., 2012). More number of both up and down regulated genes were observed during first stage of fruit infection in tolerant type (FT_1 vs. FT_C) as compared to number of up regulated genes in FS_1 vs. FS_C, FS_2 and FS_C vs. FS_3 vs. FS_C results suggest that more upregulated DEGs are involved in tolerant type and there is rapid response of tolerant type to pathogen infection as compared to susceptible type. Higher number of downregulated genes in control compared susceptible leaf and fruit samples at different stages of blight infection as compared to leaf and fruit transcriptome of tolerant types suggest downregulation of genes involved in pathways important for disease resistance like phenylpropanoid biosynthesis, which mainly carry out synthesis of lignin and flavonoids responsible for resisting pathogen infection. Similar results were obtained by Song, et al. (2019) while carying DEG analysis of *Pseudomonas syringae*

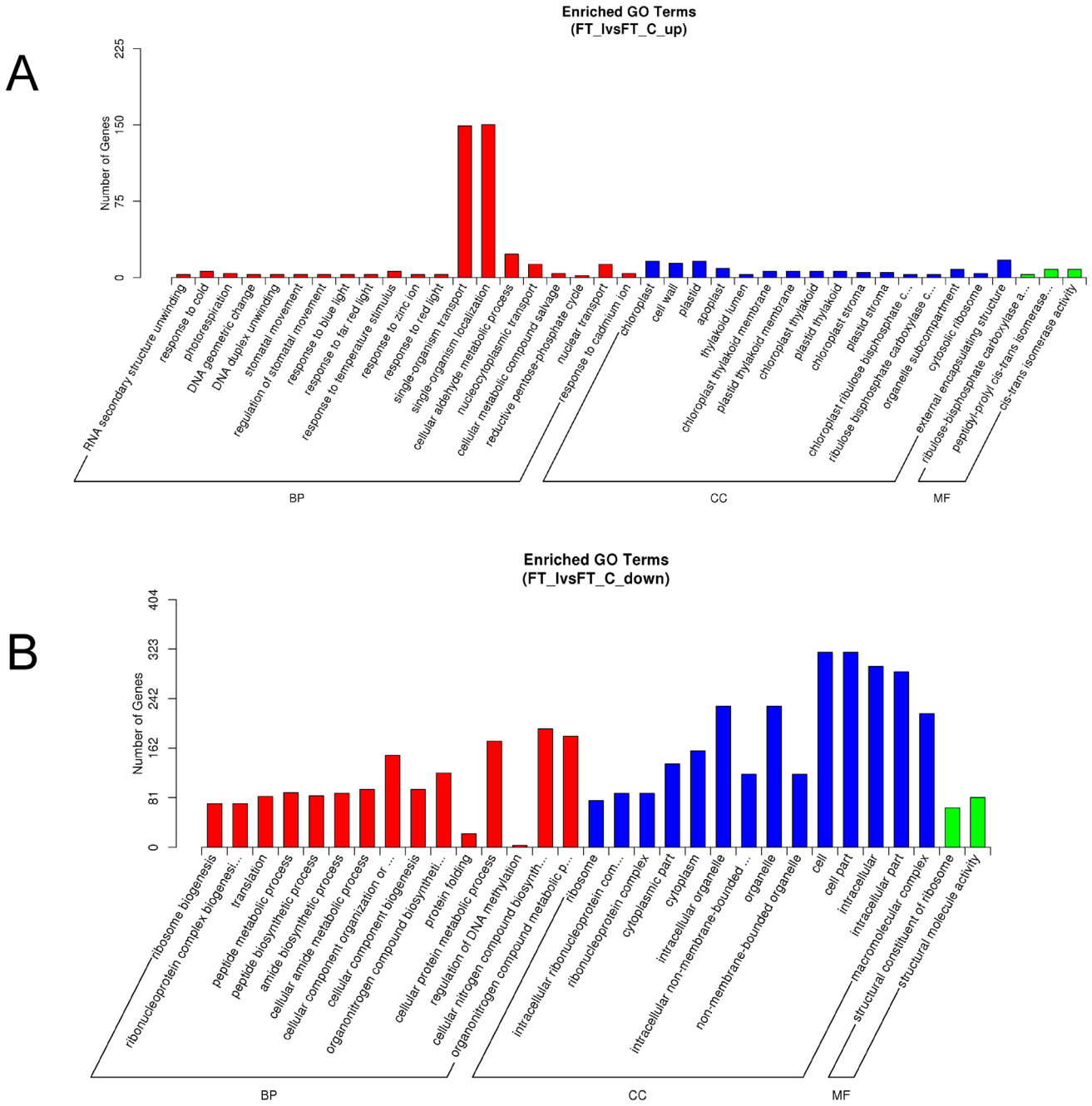

Fig. 8. (A) GO annotation of upregulated genes between the sample FT_1 vs FT_C. (B) GO annotations of downregulated genes between FT_1 vs FT_C.Y-axis represents the total number of genes in each category. X-axis represents different GO classifications of up- and downregulated genes.

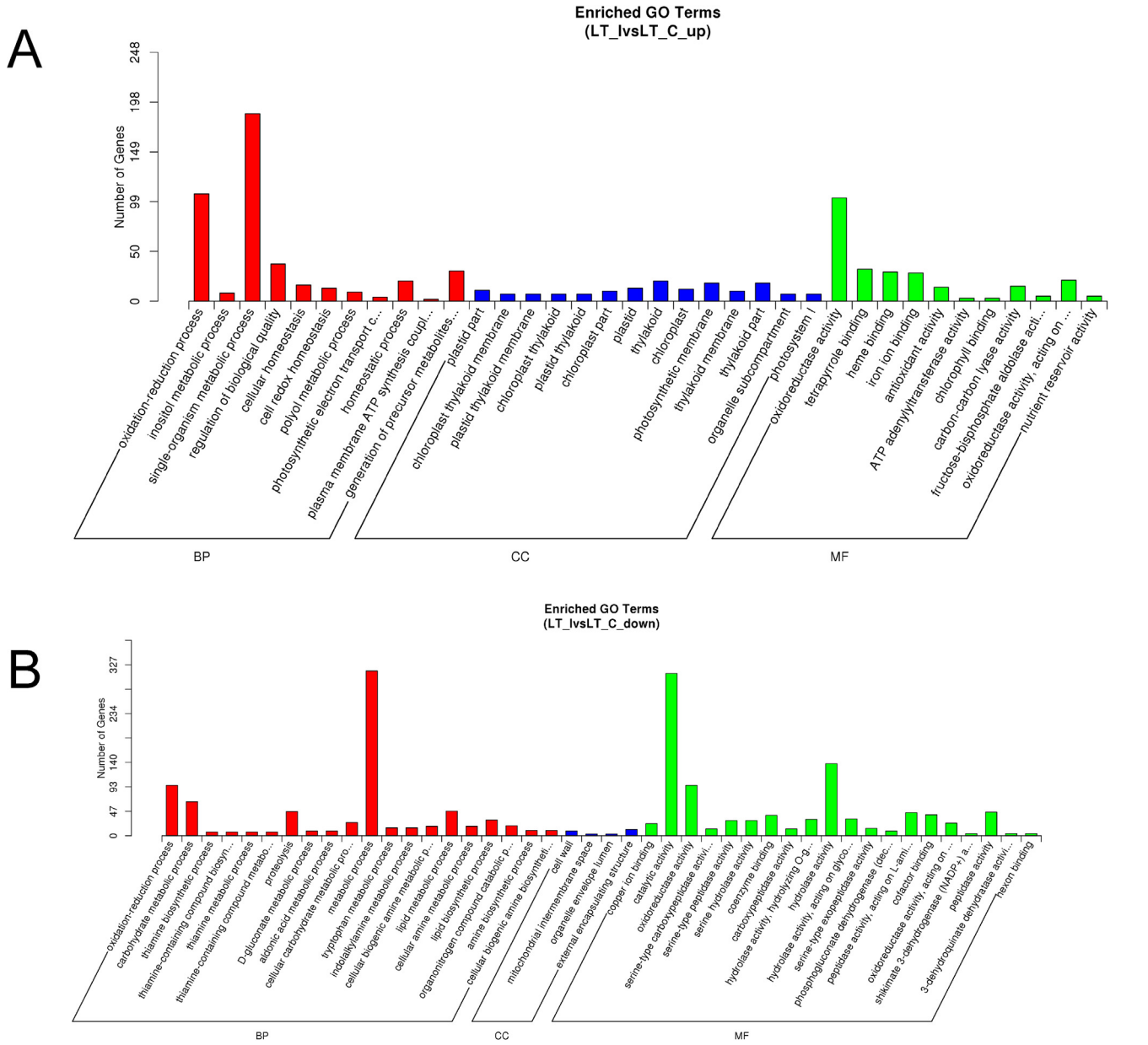

Fig. 9. (A) GO annotation of upregulated genes between the sample LT_1 vs LT_C. (B) GO annotations of downregulated genes between LT_1 vs LT_C.Y-axis represents the total number of genes in each category. X-axis represents different GO classification of up- and downregulated genes.

pv. *actinidiae* resistant and susceptible kiwifruit genotypes upon challenge inoculation.

The most enriched GO terms were related to oxidation reduction and protein binding in differentially expressed genes (Table 13). Thedownregulated genes were found to be under the spliceosome and RNA transport category (FS_1 vs FS_C, FS_2 vs FS_C, FS_3 vs FS_C) (Table 14). When the infected leaf samples of Bhagawa were compared with the control (LS_1 vs LS_C) (LC_2 vs LS_C) (LS_3 vs LS_C), most of the upregulated genes were in metabolic processes on GO analysis and downregulated genes were predominantly involved in catalytic activity (Table 13). The KEGG annotation showed that processes such as photosynthesis, phenyl propanoid biosynthesis and amino sugar/nucleotide metabolism were downregulated (Table 14). This finding agrees with previously published literature in that for the invocation of the plant defense system toward the invading pathogen, energy plays a critical role for the differential expression of several genes and pathways (Scheideler et al., 2002). The genes involved in photosynthesis and chrolophyll biosynthesis were downregulated at the time of invasion of virulent and avirulent pathogens (Denoux et al., 2008, Rojas et al., 2014, Swarbrick et al., 2006, Truman et al., 2006).

We found that when comparing LS_2 vs LS_C, along with the pathways that were downregulated in stage 1, starch sugar metabolism was downregulated. In sweet orange, *Citrus sinensis*, the starch levels were increased in leaves infected with *C. liberibacter asiaticus*, which causes citrus huanglongbing, by about 3 to 7 folds as compared with controls. The cross-talk between the pathogen and the plant may lead to the differential expression of starch metabolism genes and their downregulation (Fan et al., 2010). Similar phenomena might be exhibited by infected pomegranate, which needs further investigation.

As described in Table 8, combinations were selected for the comparative transcriptome analysis between infected leaf and fruit

Table 14
Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enrichment analysis of up- and downregulated genes among the different samples compared. NA represents the status of regulation, which is not significant as compared with the other processes of GO. The number in the brackets represents the number of genes in that process.

Samples compared	KEGG classification of upregulated genes and their number	KEGG classification of downregulated genes and their number
LT_1 vs LT_C	Photosynthesis (15), terpenoid biosynthesis (11)	Thiamine metabolism and gap junction (9)
FT_1 vs FT_C	Carbon metabolism (24), biosynthesis of aminoacid (20), protein processing in endoplasmic reticulum (38)	Ribosome (60)
FS_1 vs FS_C	Photosynthesis (35), oxidative phosphorylation (35), protein processing in endoplasmic reticulum (26)	Spliceosome (59)
FS_2 vs FS_C	Photosynthesis (36), oxidative phosphorylation (29), protein processing in endoplasmic reticulum (28)	Spliceosome (63), RNA transport (40)
FS_3 vs FS_C	Photosynthesis (36), oxidative phosphorylation (31), protein processing in endoplasmic reticulum (28)	Spliceosome (63)
LS_1 vs LS_C	Photosynthesis (12), oxidative phosphorylation (14)	Photosynthesis (16), amino sugar and nucleotide sugar metabolism (15)
LS_2 vs LS_C	Plant pathogen interaction (18)	Starch and sucrose metabolism (27), photosynthesis (24), amino sugar and nucleotide sugar metabolism (24), phenylpropanoid biosynthesis (18)
LS_3 vs LS_C	Phenylpropanoid biosynthesis (14), phenyl alanine metabolism (13)	Amino sugar and nucleotide sugar metabolism (26), starch and sucrose metabolism (23), phenylpropanoid biosynthesis (14), biosynthesis of aminoacid (20)

samples between IC 524207 and Bhagawa. While comparing FS_1 vsFT_1 and FS_2 vs FT_1, KEGG annotation showed that the carbon metabolism pathway was upregulated (Supplementary dataset 18). At the time of pathogen infection, generally carbon metabolism is high because of the upregulation of associated genes, which turns on the defense mechanisms by the activation of defense genes and finally leads to the accumulation of H_2O_2 and salicylic acid, etc. There is down regulation of pathways which play critical role in defense response against pathogen like protein processing in endoplasmic reticulum, flavanoid biosynthesis, phenylpropanoid biosynthesis, etc, when transcriptome of infected fruit samples Bhagawa was compared with infected fruit transcriptome of IC 524207, this might be one of the reasons for fast progression of blight infection in susceptible tissues (Korner et al., 2015, Song et al., 2019). The downregulation of the carbon metabolism pathway in infected leaf samples of Bhagawa when compared with infected leaf samples of IC 524207 and (LS_1 vs LT_1 and LS_2 vs LT_1) (Supplementary dataset 17 and 18) may occur because of downregulation of genes involved in photosynthesis, which alter the carbohydrate metabolism and was also observed in Potato infected with *Rhizoctonia solani* (Aliferis and Jabaji, 2012). Finally, some of the important genes that code for xyloglucan endotransglycosylase, superoxide dismutase, and alcohol dehydrogenase were validated by RT-qPCR.

5. Conclusions

We aimed to study and understand the defense response of pomegranate against the *X. axonopodis* pv. *punicae* infection, which is the sole cause of blight in pomegranate. The data from this large-scale transcriptome study by using Illumina sequencing was of high quality and the largest pomegranate transcriptome data available to date. Moreover, in-depth comparative analysis of RNA-Seq data, especially the comparison between infected leaf samples of Bhagawa with the control provides insights into the biological significance of downregulation of genes involved in photosynthesis. Similarly, comparison of infected fruit samples of Bhagawa with infected fruit samples of IC 524207 showed how the upregulation of the carbon metabolism pathway would provide resistance against the infection. This report gives information on the defense strategies adopted by the pomegranate against *X. axonopodis* pv. *punicae* and also provides the preliminary information about the differentially expressed genes between two pomegranate varieties and their possible role in tolerance/susceptibility to infection.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval and consent to participate

None.

Consent for publication

All the authors have approved the final article.

Availability of data and material

None.

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Authors' contributions

Design of the study by NVS, RKP, SP and UKR. Drafting of the manuscript and manuscript preparation by NVS, RS, DBK, RKP, PP, BK, PVPSA, UKR, and DSB. RNA sequencing and data analysis by NVRM, AT, PVPSA, HK and DSB. Sample preparation and collection of experimental materials by NVS, SP, DMM and VRS. Preparation of pure cultures of pathogen, challenge inoculation, confirmation of *Xanthomonas axonopodis* pv. *punicae* infection by JS, SP, NVS. DNA isolation, purification and quantification and qPCR validation by SKP. RNA isolation and cDNA synthesis by NVS, SKP.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2020.07.023.

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