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Integrative Analysis of the *DICER-like* (DCL) Genes From Peach (*Prunus persica*): A Critical Role in Response to Drought Stress

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DICER-likes (*DCLs*) proteins are the core component for non-coding RNA (ncRNA) biogenesis, playing essential roles in some biological processes. The *DCL* family has been characterized in model plants, such as Arabidopsis, rice, and poplar. However, the evolutionary aspect and the expression mechanism under drought stress were scarce and have never been reported and characterized in one of the most important worldwide cultivated fruit trees, peach (*Prunus persica*). Eight *DCLs* genes in the *Prunus persica* genome were detected, in addition to 51 DCLs in the other seven Rosaceae genomes. The phylogenetic analysis with *Arabidopsis thaliana* and RTL1 gene as outgroups suggested that DCL members are divided into four clades: DCL1, DCL2, DCL3, and DCL4 with several gene gain/loss events of DCL gene copies through the evolutionary tract of the Rosacea family. The number of homologous DCL copies within each clade, along with the chromosomal location indicated gene duplication event of the DCL2 gene occurred once for the subfamily Amygdaloideae and twice for *Pyrus communis* and *Prunus dulics* and trice for the *P. persica* on Chromosome number 7 genes. Another duplication event was found for the DCL3 gene that occurred once for all the eight Rosaceae species with no match in *A. thaliana*. The DCL genetic similarity and activity was evaluated using BLASTp and previously published RNA-seq data among different tissues and over different time points of peach trees exposed to drought conditions. Finally, the expression pattern of *PrupeDCLs* in response to drought stress was identified, and two of these members, Prupe.7G047900 and Prupe.6G363600, were found as main candidate genes for response to drought stress. Our data presented here provide useful information for a better understanding of the molecular evolution of *DCL* genes in Rosaceae genomes, and the function of *DCLs* in *P. persica*.

Keywords: peach, DCL genes, evolution, expression profile, duplication

INTRODUCTION

Plants are continuously exposed to several biotic and abiotic stresses during their life cycle. Among others, drought is one important abiotic stress that cause a severe loss in the agriculture sector. Over the course of evolution, plant have acquired different drought-tolerance mechanisms that lead to the emergence of new genotypes and varieties with different drought tolerance capabilities. The duration of water deficit in the soil influence the drought stress severity which initiate with water loss from the cells that leads to cellular dehydration, osmotic stress, and reactive oxygen species production (Khan et al., 2015a; Hasanuzzaman et al., 2018). Then activates various molecular pathway cascades of large signaling network and increasing the activities of antioxidant enzymes (Liu et al., 2014; Haider et al., 2018).

Peach (*Prunus persica*) belongs to the Rosaceae family, which is an important worldwide cultivated fruit tree (Verde et al., 2013; Cao et al., 2014). Because of its short reproductive cycle, nutritional and economic importance, and small genome size (Alves et al., 2016; Zhou et al., 2019; Zhou et al., 2021), *P. persica* has become an emerging model tree species for genetics, molecular biology, and plant physiology research. Under drought stress, fruit dry matter content and firmness due to the reduction of water in fruits increases, resulting in smaller fruits (Haider et al., 2018; Priya et al., 2019; Singh et al., 2019). Consequently, the fruit surface conductance and its transpiration decrease causing a significant effect on the concentrations of non-structural compounds either through the decrease in dilution and/or modifications of their metabolism (Rahmati et al., 2014). Also, drought has a critical effect on fruit growth and quality properties. Trees yield would be sharply reduced at least 25–50% in yield total weight (Rahmati et al., 2014; Khan et al., 2015b; Belal et al., 2017; Haider et al., 2018).

The small RNA (sRNA) biogenesis and regulation are controlled by three RNAi proteins-encoded gene families, known as Dicer-like (DCL), Argonaute (AGO), and RNA-dependent RNA polymerase (RDR) (Vaucheret, 2006; Chapman and Carrington, 2007; Kapoor et al., 2008; Qu et al., 2008; Cao et al., 2020b). Among them, Dicer and Dicer like (DCLs) genes are an important components of sRNA biogenesis in plants (Xie et al., 2005; Margis et al., 2006; Kapoor et al., 2008; Vaucheret, 2008; Liu et al., 2009b; Mukherjee et al., 2013). In the same context, Acquisition of DCL functions associated with distinct RNA interference branches in plants occurred during, not before, evolution of the plant lineage (Xie et al., 2005). The *DCL* gene family usually contains inconsistent number of genes copies that vary among plant species (Kapoor et al., 2008; Liu et al., 2009a; Qian et al., 2011; Cao et al., 2020b). For example, the number of recorded DCL copies was four in Arabidopsis (*Arabidopsis thaliana*), five in poplar (*Populus trichocarpa*), willow (*Salix suchowensis*) and maize (*Zea mays*), seven in tomato (*Solanum lycopersicum*), and eight in rice (*Oryza sativa*) (Finnegan and Matzke, 2003; Kapoor et al., 2008; Qian et al., 2011; Qin et al., 2018; Cao et al., 2020b). Based on previous researches, the responses of DCLs to drought, cold, and salt showed to be quite different, indicating that plants might have specialized regulatory mechanism in response to different abiotic stresses (Liu et al., 2009a). In *A. thaliana* all the DCL genes were fully characterizes, and their role in drought tolerance in addition to the sRNA biogenesis was confirmed (Xie et al., 2005; Deleris et al., 2006). However, the number and function of *DCL* genes in other plant species (e.g., Roseacea) are poorly studied (Kapoor et al., 2008; Song et al., 2012; Qin et al., 2017).

The current study aimed to characterize the DCL gene family at genomic and transcriptomic levels under drought conditions in Peach (*Prunus persica*) (International Peach Genome et al., 2013; Verde et al., 2013; Verde et al., 2017) in comparison with additional seven other Rosaceae species. Strawberry (*Fragaria vesca*) (Li et al., 2019b), China rose (*Rosa chinensis*) (Hibrand Saint-Oyant et al., 2018; Raymond et al., 2018; Li et al., 2019a; Lin et al., 2019), and Black Raspberry (*Rubus occidentalis*) (VanBuren et al., 2018) that represent the Rosoideae subfamily. Two species from genus Maleae, apple (*Malus* × *domestica*) (Bianco et al., 2014), and pear (*Pyrus communis*) (Linsmith et al., 2019), in addition to species from genus Prunus, Almond (*Prunus dulcis*) (Alioto et al., 2020), and Armenian plum (*Prunus armeniaca*) (Jiang et al., 2019), all represent the Amygdaloideae subfamily.

MATERIALS AND METHODS

Extraction and Motif Analysis of DCL Genes

The genome sequences of eight Rosaceae species (**Supplementary Table 1**) were downloaded from the GDR database.[1] The *DCLs* of *A. thaliana* (*AtDCL*) were downloaded from TAIR database[2] as described by Zhang et al. (2012), Verde et al. (2013), Wu et al. (2013), Daccord et al. (2017), Edger et al. (2018), and Cao et al. (2020b). The HMM model of DCLs was generated based on *AtDCL* copies by TBtools software (Chen et al., 2020), and then it was used to identify the DCLs members by HMMER3 software (Mistry et al., 2013). All putative genes were validated by using NCBI database[3] to confirm and acquire *DCL* hits from Viridiplantae genomes. To avoid sequence redundancy; we only considered primary transcripts in this study. The primary transcript names, chromosomal locations, and protein lengths of DCLs were obtained from the genome annotation files of these eight Rosaceae genomes. At protein level, *Prunus persica* DCL copies were investigated for conserved motifs using MEME suite online tool.[4] At coding DNA sequence (CDS) level, Similarity levels among different DCLs genes were conducted by using "Circos" plot design software[5] that basically visualized by Krzywinski et al. (2009) and described by Zhang et al. (2013) as similar genome regions.

Phylogenetic Analysis and Evolutionary Calibration

The MAFFT software was used to conduct three multiple alignments, one includes all the *P. persica* DCL copies, the other

[1] http://www.rosaceae.org/
[2] https://www.Arabidopsis.org/
[3] https://www.ncbi.nlm.nih.gov/
[4] https://meme-suite.org/meme/
[5] http://circos.ca/

includes all BLAST top 10 hits of the from NCBI database (Tophits-set) and another includes all Rosaceae-related DCL proteins (Rosa-set). The second and third alignments were performed along with the *AthDCL* copies (Katoh et al., 2005). The selected Rosaceae species relationship dendrogram was drawn based on Taxonomy database (NCBI) and visualized using iTOL webtool[6] and supported with estimated separation time obtained from[7] The Fasttree V2 software (Price et al., 2010) was used to construct the phylogenetic tree including retrotransposon-like 1 (RTL1) genes from the same selected Rosaceae species as outgroup using Jones-Taylor-Thorton model at rate categories of sites equal 20. The DCL-based phylogenetic tree was evolutionary calibrated by relative-time maximum likelihood method using MEGA-X software (Kumar et al., 2018).

RNA-Seq Expression Analyses

Two published RNAseq data were used to validate the expression of the detected DCL copies from previous studies on *P. persica*, one sampled different tissue from high and low altitudes (Leaf, Phloem, Flower, Fruit, Seed, and Root; Bioproject: PRJNA694331) and the other sampled the fruit flesh under drought stress over different time points (0 h, 3 h, 6 h, 12 h, 24 h, 3 days, 6 days, and 12 days; Bioproject: PRJNA694007). For each data set Trimmomatic was used to remove adapter sequences and low-quality sequencing reads with default parameters (Bolger et al., 2014; Cao et al., 2020b). The HISAT2 was used to align the clean reads to the masked genome (Kim et al., 2019), and the Stringtie was used to calculate the FPKM values (Fragments Per Kilobase of transcript per Million mapped reads) of DCLs in the *P. persica* reference genome (Pertea et al., 2016). The expression of DCL among different tissue was shown as a heatmap drawn by TBtools software (Chen et al., 2020). While the expression of DCL over time was modelized in expression clusters and visualized as parallel coordinate plot using xlstat software.[8]

[6]https://itol.embl.de/

[7]www.timetree.org

[8]https://www.xlstat.com/

RESULTS AND DISCUSSION

Identification of *DCL* Genes in *Prunus persica* and Other Rosaceae Species

Total of eight DCL copies were detected in *P. persica* by BLAST search (**Supplementary Table 2**). Five motifs were defined, all identified as ribonuclease III domain according to pfam database. The five motifs were distributed differently among the eight copies, causing different phylogenetic and clustering signals (**Figure 1A**). All the four DCL copies as previously reported from *A. thaliana* and other species were detected and phylogenetically clustered accordingly (Mukherjee et al., 2013; Cao et al., 2020b). The top 10 BLAST hits of the eight *P. persica* DCL copies were equally clustered into four major clusters, where DCL1 and DCL4 were monophyletic, while DCL2 and DCL3 were paraphyletic, as shown in **Figure 1B**.

In Rosaceae, 405 *DCL*-similar transcripts were detected from the seven selected Rosaceae genomes, including all isoforms. By excluding, the five DCL copies of *A. thaliana* (AT), and the eight *P. persica* (Prupe), a total of 51 non-redundant *DCLs* were retained for further analysis, five *F. vesca* (Frv), five *R. chinensis* (Rch), six *P. armeniaca* (PAR), seven *P. dulcis* (Prudul), seven *R. occidentalis* (Roc) (Xie et al., 2005), nine *Pyrus communis* (pycom), and 12 *Malus* x *domestica* (MDP) as displayed in **Figure 2A** and **Supplementary Table 2**. Compared with other families, DCL gene family contains relatively few members, which is consistent with the previous studies reported

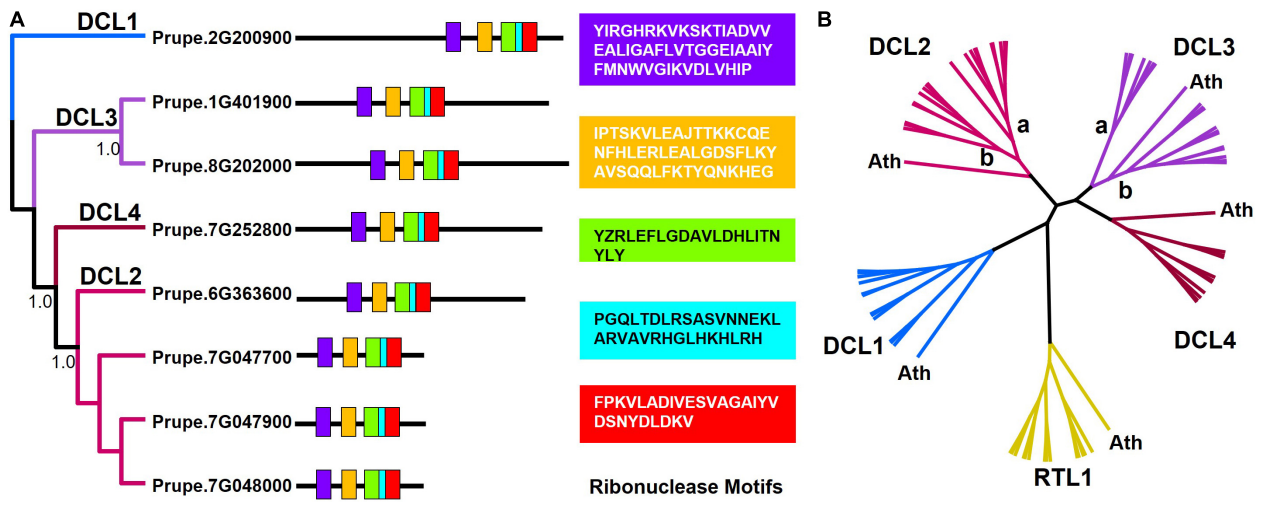

FIGURE 1 | Conserved motifs of *Prunus persica* DCLs genes. **(A)** Gene structure and domain compositions of *P. persica* DCLs based on the presence of conserved Ribonuclease motifs (detailed in colored boxes) were arranged and clustered in four groups. **(B)** Unrooted maximum likelihood DCL phylogenetic tree shows the RTL1 gene outgroup, and *A. thaliana* species outgroup along the NCBI BLAST top 10 hits of *P. persica* DCL copies. A unique cluster is observed for DCL1, and DCL4, while both DCL2 and DCL3 were separated into two subclades.

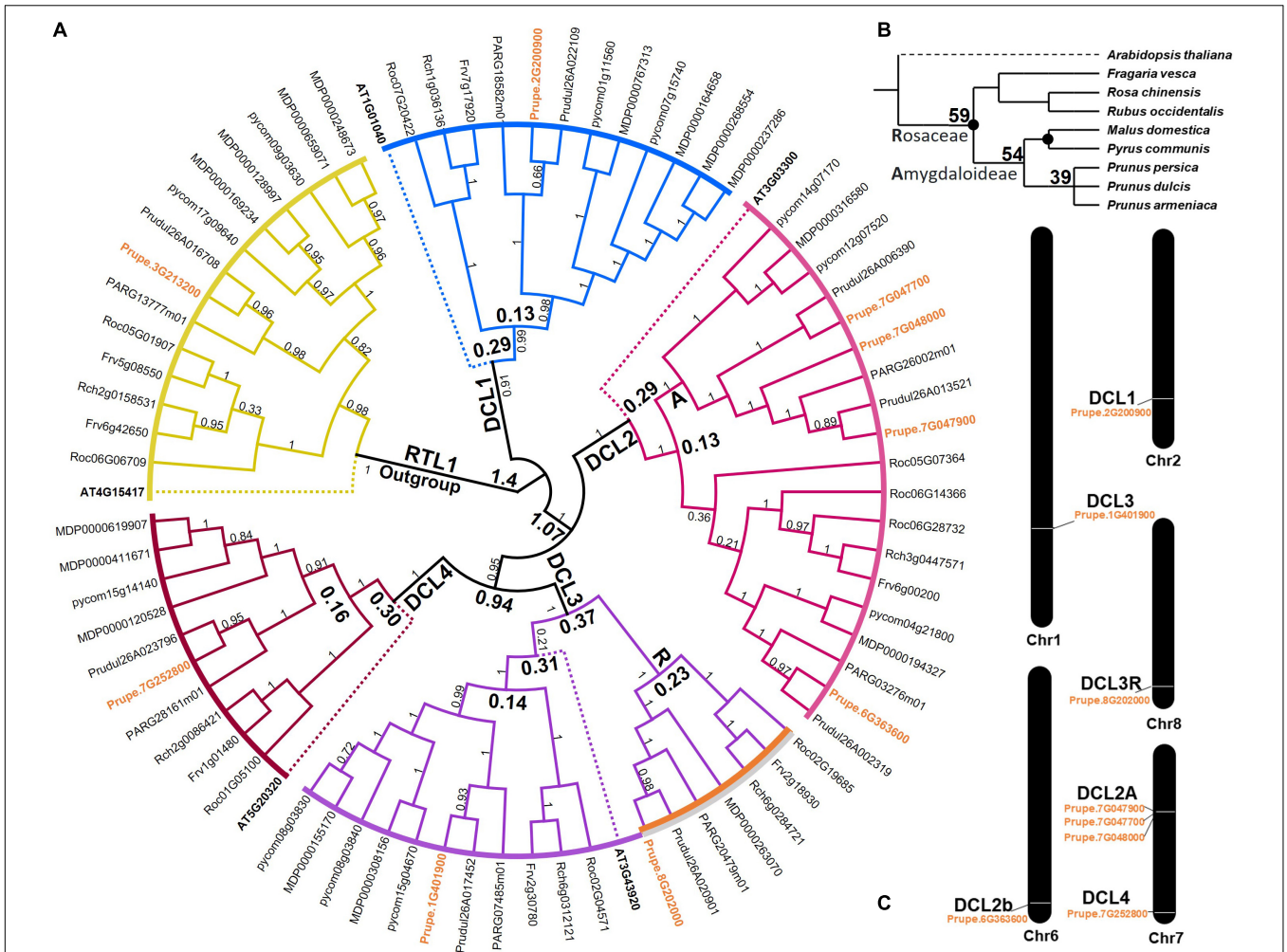

FIGURE 2 | The time-calibrated maximum liklihood phylogenetic tree of DCLs genes of eight Rosacea species. **(A)** The circular dendogram with colored clusters, each represent one DCL and the RTL1 outgroup. The *Prunus persica* DCL genes are colored with orange while Arabidopsis DCL genes are marked by dot-lines. The Rosaceae specific DCL3 copy cluster was outlined by orange-gray bolded line and named by letter (R). The Amygdaloideae specific DCL2 copy cluster is named by letter (A). **(B)** Taxonomy-based relationship of the eight Rosaceae species timely calibrated using data obtained from www.timetree.org/. Black ovals suggest whole-genome duplication (WGD) occurrences. **(C)** Distributions of DCLs genes on *Prunus persica* chromosomes. Eight DCLs genes are mapped on five out eight chromosomes. The Amygdaloideae specific DCL2 triple copies are adjacent on chromosome 8.

in *P. trichocarpa* (5) (Cao et al., 2020b), *S. suchowensis* (5) (Cao et al., 2020b), *O. sativa* (8) (Kapoor et al., 2008), *S. esculentum* (7) (Bai et al., 2012) and *A. thaliana* (4) (Deleris et al., 2006). It does not appear to be a direct correlation between the number of *DCL* genes and genome size. For example, it has been observed that the numbers of *DCL* genes differed between *P. armeniaca* (6) and *P. dulcis* (8), but their genome size is not significantly differed (*P. armeniaca*: 206.1 Mb, and *P. dulcis*: 208.9 Mb). However, *F. vesca* and *P. armeniaca* had equal number of *DCL* copies, although they have different genome size: *F. vesca* (219.29 Mb) and *P. armeniaca* (206.10 Mb). Rosaceae species share an ancient whole-genome duplication (WGD) event (Wu et al., 2013; Cao et al., 2020a), while experienced a more recent WGD event (Wu et al., 2013; Daccord et al., 2017), which might explain why *M. x domestica* contains the highest number of *DCL* gene copies among the studies Rosaceae species.

Phylogenetics and Evolutionary Analysis

All *DCL* genes were clustered into four clades: DCL1, DCL2, DCL3, and DCL4. This spread on DCLs genes indicating that they play critical roles in plant antiviral response (Necira et al., 2021), as well as contribute to the phased secondary siRNAs production (Gasciolli et al., 2005). clade DCL1 included one *PrupeDCL1*, one *FrvDCL1*, four *MDPDCL1*, two *pycomDCL1*, one *PrudulDCL1*, one *RocDCL1*, one *RchDCL1*, one *PARDCL1*, and one *AthDCL1* was set as outgroup in the DCL1 clade with the processing of 21 nucleotides (nt) miRNA and contributing to the derivation of siRNAs (Kurihara and Watanabe, 2004; Kurihara et al., 2006), as shown in **Figure 2A**. Members of the DCL2 clade contained four *PrupeDCL2*, one *FrvDCL2*, two *MDPDCL2*, three *pycomDCL2*, four *PrudulDCL2*, three *RocDCL2*, two *PARDCL2* and one *RchDCL2* in addition to one *AtDCL2* as out group. DCL2A showed tandem duplication

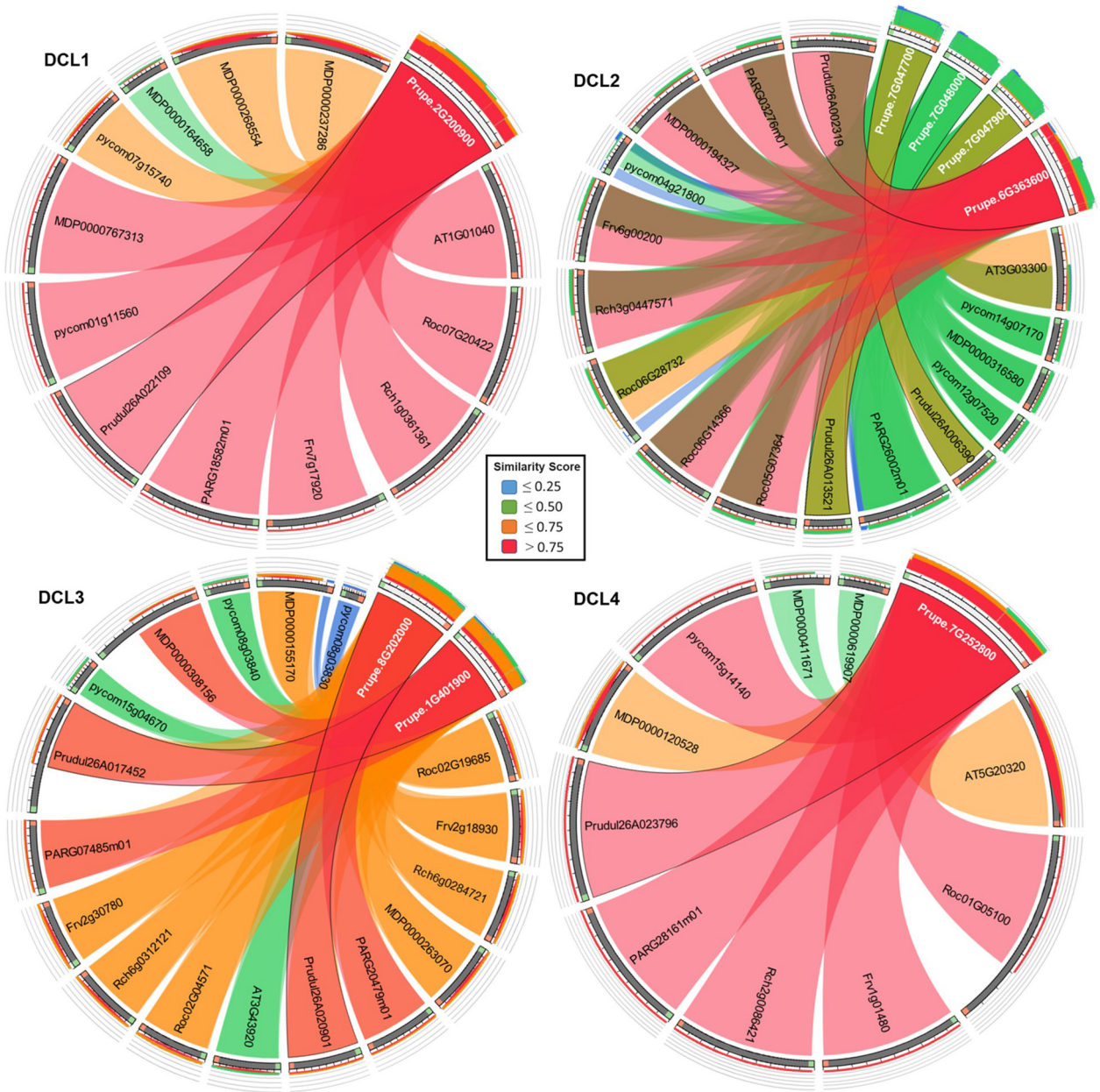

FIGURE 3 | Circos plot of protein similarity among the identified DCLs genes copies. Rosaceae DCLs genes copies were mapped using BLASTP data on the different species which were anchored to the PrupeDCLs. Similarity score is indicated on the inner box and the highlighted arcs in the center of the circos link homologous DCLs genes. *P*-value<0.05.

between both genes (Prupe.7G047700 and Prupe.7G048000). The members of the DCL3 clade included two *PrupeDCL3,* two *FrvDCL3,* three *MDPDCL3*, three *pycomDCL3*, two *PrudulDCL3*, two *RocDCL3*, two *RchDCL3,* two *PARDCL3* in addition to one *AtDCL3* as out group. Our results of phylogenetic analysis showed an important occurrence in the DCLs development of Rosaceae. Clade 3 witnessed a specific split for Rosaceae copies which separated before the Arabidopsis speciation event. That was unlike the usual occurrences in the stages of DCLs duplication of Rosaceae family (0.37 part of the separation time). This event indicates the important occurrence in the evolution of these species and may offer more insights in the coming evolution researches. These copies in clade DCL3 cluster was outlined by orange-gray bolded line and named by letter (R) in **Figure 2A**. The chronological convergence of the Arabidopsis separation event to the Rosaceae family was noticeable in the phyletic analysis as relative fragments [DCL1 and DCL2(±29); DCL3(±31); DCL4(±30)] which indicates to the normal development and synchronize speciation in different evolution events. Phylogenetic analysis for species was created

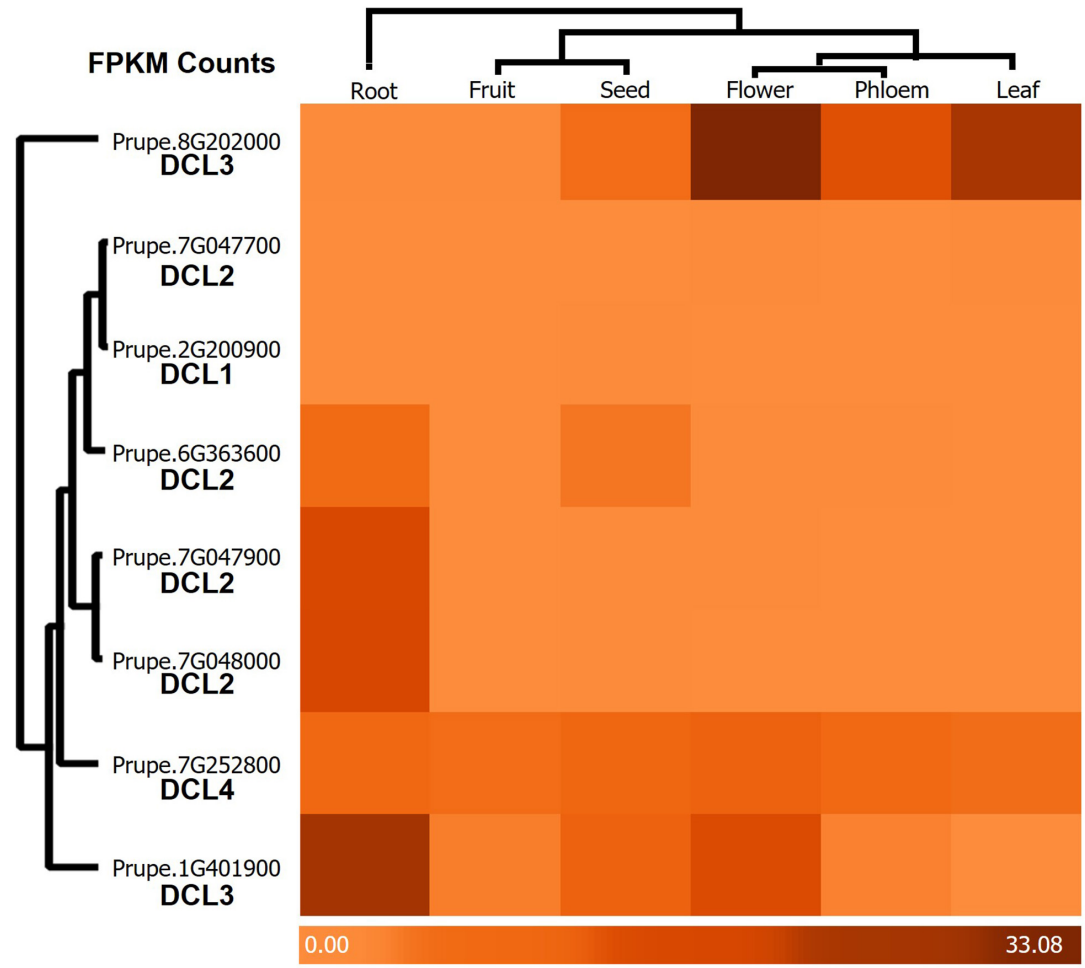

FIGURE 4 | Heatmap of tissue expression pattern of *PrupeDCLs* blast data in Root, Fruit, Seed, Flower, Phloem, and Leaf by FPKM counts. The color bar showed the expression degree. The phyletic clades were designed within FPKM values counts to identify the most relative items for displaying.

and the taxonomy-based relationship of the eight Rosaceae species timely calibrated to show the evolutionary relationship between species. The occurrence of the speciation for sub-family Amygdaloideae from Roseaceae family into two branches was demonstrated from the Rosaceae within the estimation study of the relative time 54 million years as time part of the speciation events, as noticed in **Figure 2B**. The values in the phyletic phases refer to the relative. Black ovals correspond to inferred locations of whole-genome duplication (WGD) events and the values in the head of clades refers to the approximately time of duplications per million years. The distributions of DCLs genes on *Prunus persica* chromosomes showd eight DCLs genes mapped on five out eight chromosomes as shown in **Figure 2C**. The Amygdaloideae specific DCL2 triple copies are adjacent on chromosome 7 which include the genes Prupe.7G047900, Prupe.7G047700, and Prupe.7G048000 cluster in a clade DCL2A, indicate that these genes might play different functions in siRNAs production. Previously, the functional differentiation of DCLs in one clade was found in *O. sativa DCL3* (Song et al., 2012).

DCLs Genes Similarity

Rosaceae DCLs genes copies were mapped using protein sequences of BLASTP (Altschup et al., 1990) to compare every DCLs annotated with proteome and designated the best hit as a homolog blast data on the different species which were anchored to the *PrupeDCLs*, As shown in **Figure 3**. DCL1 copies includes one *PrupeDCLs* copy (Prupe.2G200900) which scored high similarity with its duplicates while *MDPDCLs* genes copies showed less similarity than others duplicates. DCL2 has four duplicated copies of *PrupeDCLs* which have different similarity score with other DCLs. Prupe.6G363600 showed the highest similarity score with other Rosaceae copies in DCL2 compare with the other three *PrupeDCLs* copies revealed which showed low score. In DCL3, (Prupe.7G252800 and Prupe.8G202000) showed similarity with other DCLs copies of *PARDCLs*, and *PrudulDCLs* copies while *pycomDCL* (pycom08g03830) showed asymmetry with *PrupeDCL2* copies. DCL4 has one copy of *P. Persica* (Prupe.7G252800) which anchored to other copies of species and highly similarity is shown. There is a highly similarity between the DCLs copies hit with *PrupeDCLs* except *MDPDCLs*

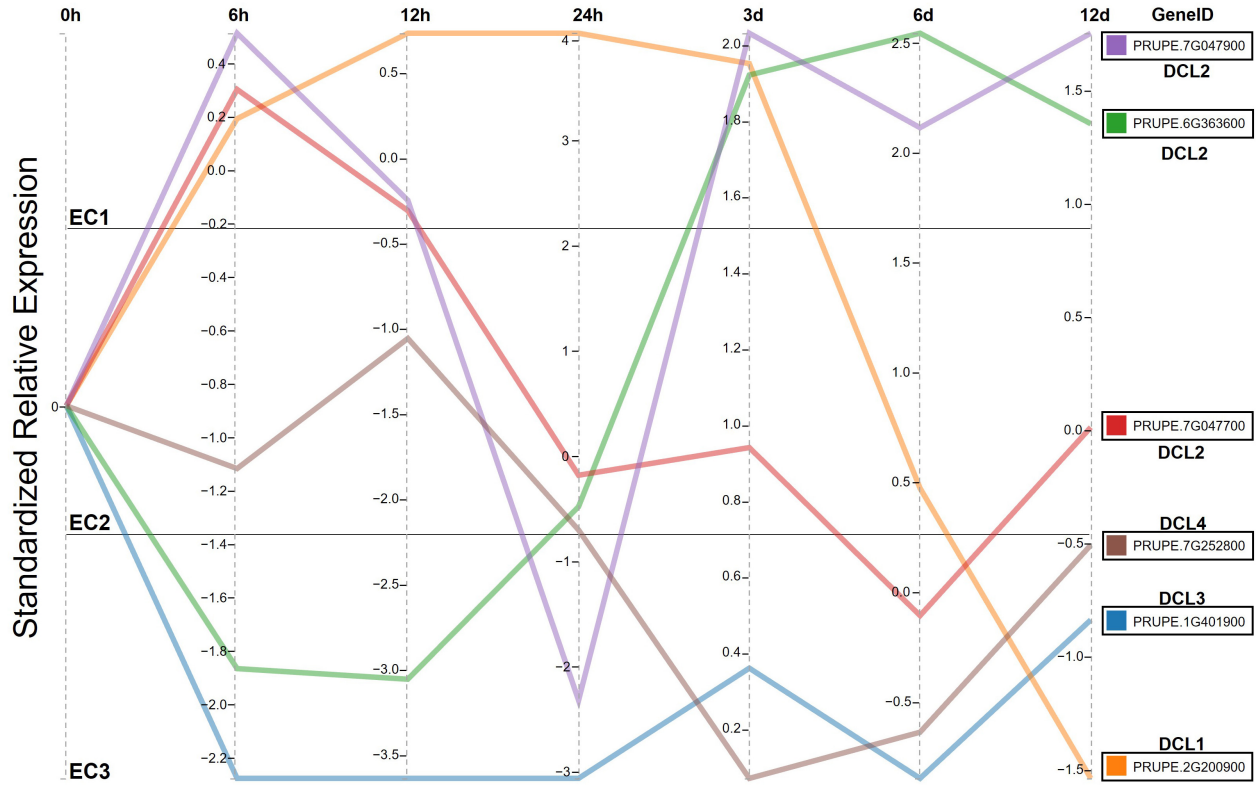

FIGURE 5 | Temporal trend for DCL genes expressed in the fruit flesh of *P. persica* exposed to drought stress over time periods: 0 h, 6 h, 12 h, 24 h, 3 days, 6 days, and 12 days stratified by 0 timing as a control. The timelines were aligned by the average of DCLs genes expression overtime by standardize relative expression. The expression area is divided into three expression clusters EC1, EC2 and EC3 to evaluate and categorize genes expressions. RNA data were used to measure the expression level of DCLs genes.

copies which have a noticeable asymmetry score ≤ 0.25. The results clarified that most of *PrupeDCLs* showed high similarity for their duplicates where this confirms the evolution path that these genes went during the species development for resistance drought.

Expression Patterns of *PrupeDCL* Genes in Various Tissues

As the master parts of sRNA biogenesis; *DCL* genes play important roles in the growth and development of plants (Fang and Spector, 2007; Song et al., 2007). The transcript levels of *DCLs* may be closely related to their physiological functions. To primarily clarify the functions of *PrupeDCLs*, the expression patterns of *PrupeDCLs* were analyzed in six tissues: root, fruit, seed, flower, phloem, and leaf. As shown in **Figure 4**, Our results found that all *PrupeDCLs* have expressed in the analyzed *P. persica* tissues, although they showed different expression patterns that provided a clear vision about how did they work. These results are consistent with the key roles of *DCL* genes in the miRNAs biogenesis that are involved in plant stress responses and development. Our results exhibited lowest expression showed by *PrupeDCL1* (Prupe.2G200900) and *PrupeDCL2* (Prupe.7G047700) in the different tissues. Other two *PrupeDCL2* genes (Prupe.7G047900, and Prupe.7G048000) showed same expression pattern degree in root tissue. It was observed that *PrupeDCL2* (Prupe.6G363600) exclusively expressed in both root tissue and seeds while *PrupeDCL4* almost identically expressed in all tissues.

Consistently, we also identified two *PrupeDCL3;* (Prupe.8G202000 and Prupe.1G401900). The results showed that Prupe.1G401900 was highly expressed in almost all tissues especially in root, and Prupe.1G401900 showed a high level of expression in flower, leaf, phloem and seeds respectively, but recorded low expression in both root and fruit.

Expression Patterns of *PrupeDCLs* Under Drought Stress

Environmental stress may influence plant growth and health by affecting the regulation of crucial plant genes (Chinnusamy and Zhu, 2009). Many stress-related genes are induced to express under external environmental stresses to help plants cope with adverse conditions (Chinnusamy and Zhu, 2009; Priya et al., 2019). For example, *DCLs* from *P. trichocarpa, S. suchowensis*, *Saccharum spontaneum,* and *Solanum tuberosum* contribute to plants resist abiotic stress (Esposito et al., 2018; Cao et al., 2020b; Cui et al., 2020). The *P. persica* is generally widely grown in irrigated semi-arid and arid regions. However, the aggravation of drought caused by the global greenhouse effect

has greatly restricted the growth and development of *P. persica*. To determine the effect of *PrupeDCL* genes on drought stress, we investigated the expression pattern of *PrupeDCL* genes in *P. persica* within the relative expression time. As regards drought stress, the results cleared that all *PrupeDCLs* were differentially expressed. The expression level of *PrupeDCLs* have different expression in both of brief and chronical exposure for drought conditions, as shown in **Figure 5**. DCL1 (Prupe.2G200900) showed high and stable expression in the short-term treatment (time 0:3 days) but it dramatically dropped at the chronic period (3 days: 12 days) while *PrupeDCL2* copies (Prupe.7G047900 and Prupe.6G363600) recorded various expression in the short-term treatment but achieved the highest expression in the chronic time of drought treatment as appeared in expression cluster EC1. On the other side, *PrupeDCL3* (Prupe.1G401900) and *PrupeDCL4* (Prupe.7G252800) have low expression in the relative time of drought treatment in EC3. That could be explained that they worked as chronic co-expression genes to resume resistance drought conditions. *PrupeDCL2* (Prupe.7G047700) positively expressed in the brief time (0–12 h) and then continued as moderate expression gene as shown in EC2 was increased at 0–24 h. The transcript levels of *PrupeDCLs2* (Prupe.7G04790 and Prupe.6G363600) were significantly upregulated by drought stress for 3–12 day, whereas the two genes from the same clade *PrupeDCL2* were unchanged, suggesting that these genes from the same ancestor were functionally redundant or neo-functionalization during evolution.

Our results provided insights into the molecular evolution of *PrupeDCL* genes in Rosaceae, and the probable roles in response to drought stresses in *P. persica.*

CONCLUSION

Although DICER-like (DCL) genes have been studied because of their critical roles in plant resistance to the abiotic stresses, the focus remains on their role in plants of the Rosaseae species, especially peach (*Prunus persica*), which needs in-depth studies to clarify their roles, and evolution in Rosaceae. In our study, it was identified about 59 *DCLs* in eight Rosaceae genomes, 8 of these genes were from *P. persica*. Based on the phylogenetic analysis, and comparison with homologs from *A. thaliana*, the *DCL* family members were divided into four clades: DCL1, DCL2, DCL3, and DCL4. RNA-seq data indicated that the recent WGD might have driven the expansion of *DCLs* in *Prunus Persica* and revealed that the identified *PrupeDCLs* play a pivotal role in different tissues development and drought stress. Overall, our data constitute a foundation for further studies examining the complexity and functioning of Rosaceae *DCL* genes in the future.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

YH and YC planned and designed the experiments. MB, YZ, ZX, and ME performed the experiments and the sequence analysis. YC and MB wrote the manuscript. YH revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.923166/full#supplementary-material

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