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Original article

In Silico analysis of *Vitis vinifera* Cabernet Sauvignon TOR and its responses to sugar and abscisic acid signaling

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ABSTRACT

Target of rapamycin (TOR) is a very critical protein in plants, which connects with biological process, glycometabolism, life span, and photosynthesis. Here, the evolutionary relationship, conserved motif, gene structure and cis-acting elements of TOR were analyzed. Promoter cis-acting elements analysis indicated various cis-acting elements respond to light, auxin, ABA and multiple signal pathway. Transcriptome sequencing and the co-expression network of *VvTOR*, sugar and abscisic acid (ABA) related genes from *Vitis vinifera* L. Cabernet Sauvignon berries indicated that *VvTOR* might participate in sugar and ABA signaling. The expression of *VvTOR* in grape suspension cells analyzed by quantitative real-time PCR showed that *VvTOR* responded to ABA and glucose treatment. These results predicted the potential functions of *VvTOR* in glucose metabolism and ABA signal pathway.

Keywords: target of rapamycin, in silico analysis, grape, sugar, abscisic acid

Introduction

TOR (target of rapamycin) is a large protein (~280 kDa) that belongs to an atypical serine-threonine protein kinase (PK), closely relates to the phosphatidylinositol 3-kinase-related protein kinase (PIKK) family and shares 40 % - 60 % identity in their primary sequence conserved from yeasts to plants and humans (Loewith & Hall 2011; Robaglia *et al*. 2012; Aramburu *et al*. 2014; Maegawa *et al*. 2015; Xiong & Sheen 2015; Dobrenel *et al*. 2016). TOR signaling network is a central metabolic network in all eukaryotes, which coordinates cell growth and development in response to all kinds of signals, including light, auxin, glucose, amino acid (Wullschleger *et al*. 2006; Xiong *et al*. 2013; Xiong & Sheen 2015; Dobrenel *et al*. 2016; Inaba & Nagy 2018). Recently, more and more research about TOR protein has been done. So far, every eukaryote genome has been examined containing the *TOR*, including yeast, plant, animal, algae, and slime mold etc. Comparing with yeast genome which has two different *TOR* genes, most plants, animals and human genomes have only one *TOR* gene, expect that two *TOR* genes were found in *Glycine max*, *Populus trichocarpa* and *Brassica rapa* (González & Hall 2017; Shi *et al*. 2018; Jamsheer *et al*. 2019). In mammals, there is only one copy of *TOR* gene, but it forms two TOR complexes, called mTORC1

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(mammalian TOR complex 1) and mTORC2 (mammalian TOR complex 2), which are formed by different elements and functionally specified proteins (Van Leene *et al*. 2019). In plants, TOR exists as only TORC1, while the TORC2 is absent, which is a key evolutionary difference between plants and mammals (Xiong & Sheen 2015; Dobrenel *et al*. 2016; Van Leene *et al*. 2019). However, it is possible that there are other undiscovered special TOR complexes in plants, or the plants TORC1 possesses partial function of mammalian TORC2 which can replace the whole function of mammalian TORC2 (Jamsheer *et al*. 2019).

Grapevine (*Vitis vinifera* L.) is considered to be one of the major fruit crops in the world. The yield of *grapes* is very abundant, and the economic value is tremendous. Grape can be used not only for wine but also for fresh fruit, dried fruit, and for grape juice. The quality of grapes depends on the accumulation of sugar to a large degree, including glucose, fructose, sucrose and so on. Sugar not only supports energy for plant growth, but also a critical signaling molecule. TOR and abscisic acid (ABA) are pivotal protein and hormone about sugar metabolism in plants (Ciereszko 2018; Rodriguez *et al*. 2019). ABA increases carbon allocation in different organs of grapevine plants by inducing accumulation of non-structural carbohydrates in leaves, enhancement of phloem area and expression of sugar transporters (Murcia *et al*. 2016). The glucose-TOR crosstalk controls many genes that are uniquely required for plant growth, defense or communication to promote fitness, adaptation and survival (Xiong *et al*. 2013). In a summary, TOR has critical influence on the sugar and ABA signal pathway, and the in silico analysis is able to provide references and theoretical basis for the further study of TOR.

In this study, in silico analysis about phylogenetic tree construction, gene structure analysis, conserved motif analysis and cis-acting elements prediction of *TOR* were performed to further understand the potential functions of *TOR*. At the same time, the study selected post-flowering 30-day, 70-day and 90-day development stages of grapevine berries Cabernet Sauvignon and extracted RNA for transcriptome sequencing. The co-expression network of *Vitis vinifera TOR* (*VvTOR*), sugar and ABA related genes reveals that *VvTOR* has a close relationship and works together with a variety of sugar and ABA metabolic genes. Furthermore, the expression of *VvTOR*, sugar and ABA related genes from the co-expression network were analyzed by transcriptomic analysis, which reveals that the early stage of grapevine berries development has a big difference with the middle and later stage. According to quantitative real-time PCR analysis, we detected the expression levels of *VvTOR* in grape suspension cells with sugar and ABA treatments to explore the roles of *VvTOR* in sugar metabolism and ABA signal pathway. Based on the above analyses of *VvTOR* gene, the study also played analysis for *VvTOR* by online analysis software, including basic physicochemical properties, hydropathicity and hydrophobicity, transmembrane structure, protein secondary and tertiary structure prediction. In conclusion, the study told us that TOR was a highly conserved protein and *TOR* promoter sequence contained multiple cis-acting elements. ABA and sugar signals could affect the expression of *VvTOR*, which implied that *VvTOR* could participate in sugar metabolism and ABA signal pathway. We expect this work could provide some references for improving the sugar content by the *VvTOR* gene in grapevine berries and offer some viewpoints for exploring the mechanism of the *VvTOR* metabolism network.

Materials and methods

Phylogenetic tree construction

Twenty-one gene and protein sequences of *TOR* from plants were downloaded from the NCBI (https://www.ncbi.nlm.nih.gov/) databases (Tab. S1). The statistical method of Neighbor-Joining was applied to construct the *TOR* phylogenetic tree by Mega 7.0. The evolutionary distances were computed using the Maximum Composite Likelihood (Saitou & Nei 1987; Huang *et al*. 2020).

Conserved motif and gene structure analysis

The motif analysis of twenty-one protein sequences of TOR was performed in the MEME (http://meme-suite.org/tools/meme). The conserved motifs were screened and visualized with TBtools (Toolbox for Biologists) v0.664435 (Chen *et al*. 2020). Gene structure analysis of twenty-one *TOR* genes was combined with a phylogenetic relationship. The genome information was from NCBI.

Cis-acting elements prediction

To further understand the potential functions of *TOR*, online analysis of 2,000 bp promoter sequence in the coding-sequence (CDS) was performed by PlantCare website (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) for cis-acting prediction (Lescot *et al*. 2002; Huang *et al*. 2020). The information about the sequence was downloaded from NCBI.

Transcriptome Sequencing

The grapevine berries Cabernet Sauvignon at 30, 70 and 90 days after bloom were collected from Beijing Lainberg International Winery (116.218778, 39.790114) and transcriptome sequencing of these berries was performed. Total RNA of grapevine berries was extracted using the RNAprep Pure Plant kit (TIANGEN, Beijing, China) and checked for a RIN number to inspect RNA integrity by an Agilent Bioanalyzer 2100 (Agilent technologies, Santa Clara, CA, US). Qualified cDNA library was constructed and applied for following sequencing (Illumina Hiseq 2000). The original

image file obtained was subjected to base recognition and error filtering. The sequenced fragments called "Reads" were obtained for analysis. In order to eliminate the influence of gene lengths and sequencing discrepancies on gene expression, the reads were converted into FPKM (Fragments per Kilobase of exon model per Million mapped Reads) for standardization of gene expression (Mortazavi *et al*. 2008). The number of fragments for each gene was counted by HTSeq and applied normalized by TMM (trimmed mean of M values) method, FPKM value of each gene was calculated using perl script (Robinson & Oshlack 2010; Anders *et al*. 2015).

Co-expression network analysis of VvTOR and sugar and ABA related genes

The co-expression networks of *VvTOR* and sugar (Glucose, Fructose, Sucrose, Glucan, Starch, Xylan) and ABA related genes were constructed according to the HRR (highest reciprocal rank) method, respectively. Sugar and ABA related genes (Tabs. S2, S3) were selected by the information of the gene description in transcriptome sequencing results and NCBI. Correlation between two genes was calculated by pearson correlation coefficient (r), using R Development Core Team (2012) version 3.5.3 parameters for HRR30 gene correlation coefficient calculation and threshold screening followed by visual analysis using Cytoscape software (Smoot *et al*. 2011; Yong *et al*. 2018).

Analysis of VvTOR Gene and Protein

The ProtParam tool (https://web.expasy.org/protparam/) was applied for analyzing the physicochemical properties of proteins. ProtScale (https://web.expasy.org/protscale/) was applied to hydropathicity and hydrophobicity analysis of proteins. TMHMM Server v.2.0 (http://www.cbs.dtu.dk/services/TMHMM/) was used for protein transmembrane structure analysis. SOPMA was applied for protein secondary structure prediction, following the link (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html). PredictProtein (https://www.predictprotein.org/) had the same function with SOPMA. However, the PredictProtein function was more comprehensive. Swiss-Model Workspace (https://swissmodel.expasy.org/) was used for tertiary structure analysis of proteins. SignalP-5.0 software (http://www.cbs.dtu.dk/services/SignalP/) could predict it if there was signal peptide.

Grape suspension cells preparation and treatments by sugar and ABA

The calli of grape cultivar Cabernet Sauvignon was cultured at 20 days interval on Gamborg B5 medium containing 0.2 mg L^{-1} 6-furfurylamino-purine (KT) and 0.1 mg L^{-1} 1-naphthaleneacetic acid (NAA) at 25 °C in 16 h of light and 8 h of darkness. Appropriate amount of grape suspension cell was cultured in B5 liquid medium at the condition of 25 °C, 110 r/min and cultured at 7 days interval. Four days after cultured, glucose (Glu, 100 mM), sucrose (Suc, 100 mM), fructose (Fru, 100 mM), ABA (10 μM), Glu (100 mM) + ABA (10 μM), Suc (100 mM) + ABA (10 μM), Fru (100 mM) + ABA (10 μM) was added into suspension cell and light culture for 12 h. Mannitol (100mM) treatment was applied as a control to exclude the effects of osmotic stress (Song *et al*. 2010). After 12 h treatment, cell was vacuum filtered, frozen in liquid nitrogen immediately and stored at -80 °C for subsequent quantitative real-time PCR analysis.

Quantitative real-time PCR analysis

To evaluate the expression levels of *VvTOR* in the suspension cells with different sugar and ABA treatments, quantitative real-time PCR (qRT-PCR) analysis was performed. Total RNA was extracted from grape suspension cells with ABA and sugars treatment using a RNAprep Pure Plant kit (CWBIO, Jiangsu, China). First-stranded cDNA was synthesized from 4 μg RNA using a HiFiScript cDNA Synthesis kit (YEASEN, Shanghai, China) and applied as a template for qRT-PCR. Dissociation curves of qRT-PCR reaction were analyzed for the specificity of primers. qRT-PCR analysis was run on CFX96 Real Time PCR System (Bio-rad, America) using UltraSYBR mixture (CWBIO, Jiangsu, China). The housekeeping gene *VvACTIN* (XM_002282480) with nearly constant expression level under all experimental conditions was applied as an internal control (Wang *et al*. 2017). The relative expression of *VvTOR* under different sugar and ABA treatments was measured according to the method of $2^{-\Delta\Delta Ct}$ (Livak & Schmittgen 2001).

Statistical analysis

Each experiment was replicated three times, and the mean ± standard deviation (SD) was reported. Statistical differences between means were evaluated by SPSS 20.0 software. Univariate analysis of variance (ANOVA) and Duncan's test were applied to establish the significance at $P < 0.05$.

Results

Phylogenetic tree, conserved motif and gene structure analysis

Phylogenetic tree of *TOR* was shown in Fig. 1A. It could be observed that *Vitis vinifera* has close ties of consanguinity with *Theobroma cacao*, *Herrania umbratical*, *Ricinus communis* and *Jatropha curcas*. The amino acid conserved domain analysis of TOR protein sequences was carried out in the online MEME program. All TOR contained conserved motifs 1 to 30 and the motifs are in the same order, except that

Raphanus sativus starts with one more motif 20 in the 5' of the sequence (Fig. 1B). This is corresponding to the viewpoint that TOR is highly conserved. The gene structure map showed the number of intron-exons in all members of TOR genes (Fig. 2A). Almost all *TOR* genes have 57 exons and there is a big difference in the full length of *TOR* genes. *VvTOR* gene is very long in the gene structure map. In close ties of consanguinity, *TOR* genes are about the same length, like *Raphanus sativus*, *Brassica oleracea*, *Capsella rubella* and *Arabidopsis thaliana*.

Cis-acting elements prediction

Analysis of cis-acting elements in the *TOR* promoters provided the basic for the understanding of potential regulation mechanism of TOR. Promoter element analysis illustrates that TOR contains multiple light responsive regulatory elements (GT1-motif, 3-AF1 binding site, AE-box, AAAC-motif and so on). Some cis-acting elements were associated with auxin (TGA-element, AuxRR-core), gibberellin (TATC-box, p-box, GARE-motif), abscisic acid (ABRE), methyl jasmonate (TGACG-motif, CGTCA-motif), salicylic acid (TCA-element, SARE). Some cis-acting elements respond to low-temperature (LTR), defense and stress (TC-rich repeats) and phytochrome down-regulation expression, while some cis-elements were involved in anaerobic induction (ARE), meristem expression (CAT-box), differentiation of the palisade mesophyll cells (HD-zip 1), MYB binding site (MRE, MBS, MBSI), MYBHv1 binding site (CCTTA-box), anoxic specific inducibility (GC-motif), circadian control (circadian) and the like (Fig. 2B). The TOR promoter contained a large number of hormone and stress response elements, indicating that TOR may plays critical role in hormone signal transduction and environment stress. Almost all TOR promoters can respond to light and anaerobic induction. *VvTOR* promoter responded to light, auxin, abscisic acid responsive, anaerobic induction and MYB binding site, which implies that *VvTOR* plays a role in these signal pathways.

Co-expression network and expression analysis of VvTOR and sugar and ABA related genes

The relationship between *VvTOR* and sugar and ABA related genes was explored by co-expression network analysis, respectively. *VvTOR* is co-expressed with 40 sugar related genes, including 13 glucose, five sucrose, nine xyloglucan, 10 glucan, one starch, two hexoses related genes (Fig. 3A). *VvTOR* is co-expressed with 28 ABA related genes (Fig. 3B). The co-expression network analysis indicates that *VvTOR* has a close relationship with sugar metabolism and ABA signal pathway. In order to understand the role of *VvTOR*, sugar and ABA related genes in the growth and development of grape berries of three stages (DAB30, DAB70, DAB90) were selected for RNA-Seq analysis. The expression levels of *VvTOR*, sugar and ABA related genes which were in the co-expression network were performed by FPKM. In contrast, *VvTOR* expressive levels are high in DAB70 and DAB90 (Fig. 4). ABA related genes expression levels were higher in DAB30, which is the opposite with *VvTOR*. The majority of sugar related genes have higher expression levels in DAB70, like sucrose, glucan, xyloglucan, hexose related genes. At the same time, xyloglucan related genes expression levels were high in DAB30 and sucrose related genes expression levels were high in DAB90 too. Glucose related genes had expression in all three stages of grape. The columns of the heat map were clustered and the result showed that the expression levels of *VvTOR*, sugar and ABA related genes in DAB70 and DAB90 were more similar compared to that in DAB30.

Expression of VvTOR with sugar and ABA treatments

In order to explore the roles of *VvTOR* in the sugar metabolism and ABA signal pathway, grape suspension cells were treated with different sugar and ABA, the expression levels of *VvTOR* in suspension cells with sugar and ABA (mannitol, Glu, Suc, Fru, ABA, Glu + ABA, Suc + ABA, Fru +

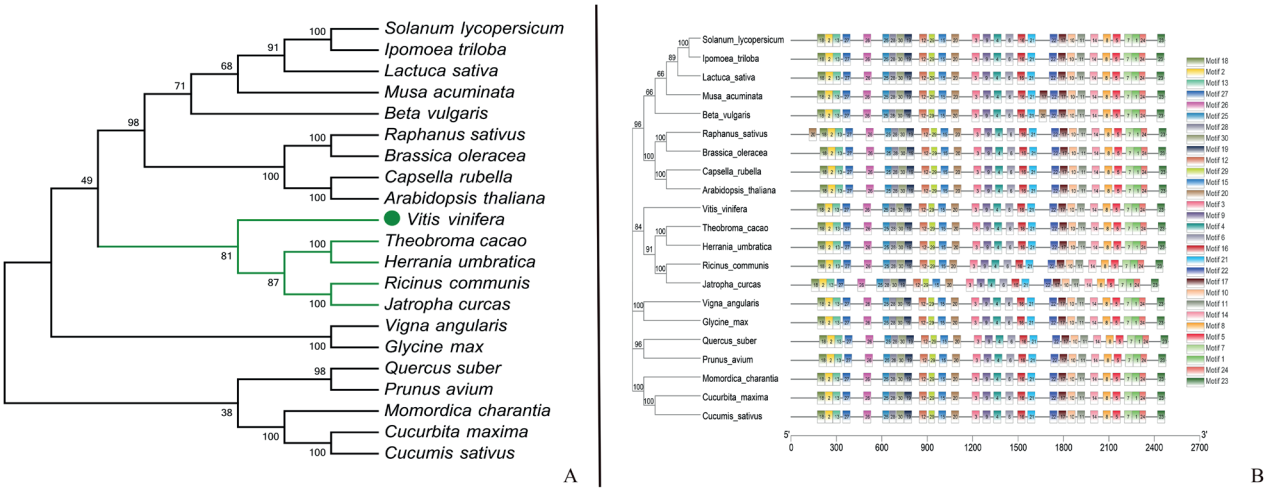

Figure 1. Phylogenetic tree and the conserve motif of *TOR*. (A) Phylogenetic tree of *TOR*. (B) The conserve motif of *TOR*.

ABA) were detected by quantitative real-time PCR. *VvTOR* responded to different sugar and ABA treatments (Fig. 5), while the expression of *VvTOR* in suspension cells with signal sugar and ABA treatment differed from that with sugar and ABA complex treatments. *VvTOR* was able to respond to Glu and ABA signals. The expression of *VvTOR* with Glu treatment was lower than mannitol treatments and ABA treatment could improve the expression of *VvTOR*. The relative expression of *VvTOR* was strongly induced by ABA+Glu. Compared with mannitol treatments, *VvTOR* was down-regulated by Suc or Fru and up-regulated by Suc + ABA or Fru + ABA, even though the difference is not significant.

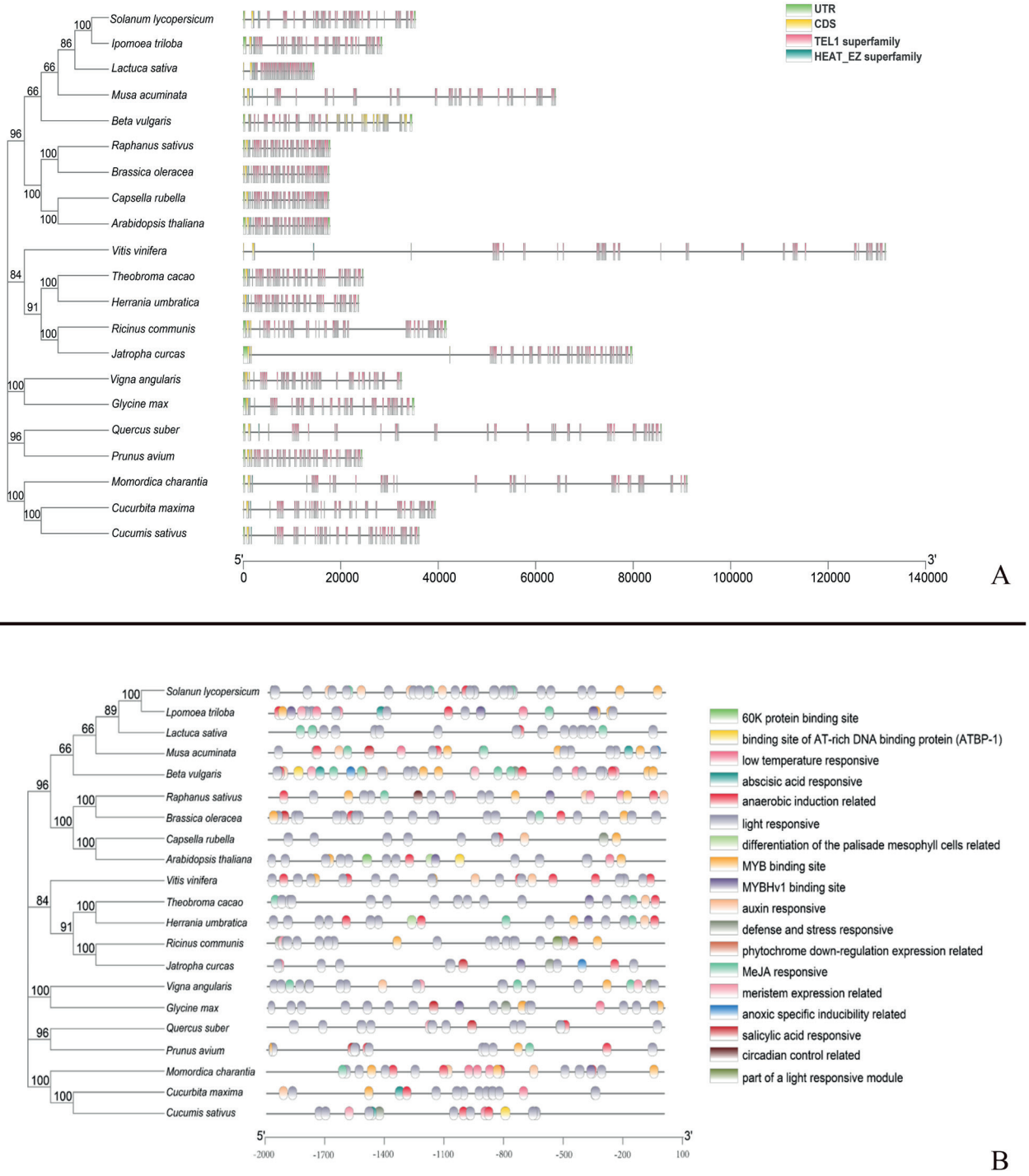

Figure 2. Gene structure analyses and the cis-acting elements prediction of *TOR*. (A) Gene structure analyses of *TOR*. The gray line indicated intron, while the green and yellow boxes indicate UTR and CDS, respectively. TEL1 superfamily and HEAT_EZ superfamily are conserved domains of TOR. (B) The cis-acting elements prediction of *TOR*.

Analysis of VvTOR Gene and Protein

The number of amino acids of *VvTOR* are 2,469. Molecular weight is 277,335,89 Da. Theoretical protein isoelectric point (pI) is 6.40. High amino acids composition is Leu 13.0 %, Ala 10.0 %, Arg 7.4 %. A protein whose instability index is smaller than 40 is predicted as stable, a value above 40 predicts that the protein may be unstable (Wilkins *et al*. 1999). *VvTOR* protein instability index is 43.51, which classifies the protein as unstable. Grand average of hydropathicity (GRAVY) is -0.099. The score is less than 0, which represents hydropathicity. The score is greater than 0, which represents hydrophobicity (Wilkins *et al*. 1999). Hydropathicity and hydrophobicity analysis of *VvTOR* protein shows that the 127th amino acid is the highest, with a score of 2.733 and hydrophobicity is the strongest. The 548th is the lowest, with a score of –2.989 and the hydropathicity is the strongest. In total, the numbers and scores of hydropathicity are greater than hydrophobicity (Fig. S1). Above all, we predict that *VvTOR* protein is hydrophilic, which it accords with GRAVY. TMHMM Server v.2.0 used for protein transmembrane structure analysis shows that all amino acids are outside. *VvTOR* is an outer membrane protein without transmembrane structure (Fig. S2). SignalP-5.0 is used for predicting if there are signal peptides in the *VvTOR* protein. Signal Peptide (Sec/SPI) likelihood is 0.0005. The closer of the signal peptide probability is to 1, the higher the probability that the protein has a signal peptide; hence, we can draw the predicted result that there is no signal protein (Fig. S3). The result corresponds to the study of hydrophilic and hydrophobic analysis and transmembrane structure analysis of proteins (Figs. S1, S2). SOPMA for protein secondary structure prediction shows that alpha helix, random coil, extended strand and beta turn are 63.57 %, 27.44 %, 5.51 % and 3.48 %, respectively (Combet *et al*. 2000) (Fig. S4). Alpha helix is the main in the secondary structure of *VvTOR* protein. Swiss-Model Workspace builds the tertiary structure model of *VvTOR* protein automatically (Fig. 6).

Discussion

TOR is a critical conserved protein to sense and integrate cellular status information from numerous stimuli, including hormone signals, nutrient and energy availability, and stress information. Although TOR protein is high conserved (Fig. 1A), the full-length of *TOR* genes varies greatly (Fig. 2A). The full-length of *TOR* genes are similar in the majority closely related species (Figs. 1A, 2A). In our experiments, *VvTOR* was co-expressed with 40 sugar genes and 28 ABA related genes, which implies that *VvTOR* may play a critical role in ABA and sugar metabolism (Fig. 3). *VvTOR* can respond to Glu and ABA signals, which is according to other researches (Xiong & Sheen 2012; Fu *et al*. 2020) (Fig. 5). Recently, scientists found that glucose can activate TOR protein further to promote the development of root hair in *Arabidopsis* (Xiong & Sheen 2012; Van Leene *et al*. 2019). *TOR* has a cis-acting element about meristem (Fig. 2B). Sugar signals can be translated by protein kinase complex, which regulates energy metabolism. *VvTOR* promoter sequence had the ABA responsive elements (Fig. 2B). Down-regulated TOR signaling by chemical inhibitor AZD-8055 also activates genes involved in stress hormone (*e.g*., ethylene, jasmonic acid (JA), and ABA) signaling pathways (Fu *et al*. 2020). Some studies suggest that two important mediators of ABA signaling, YAK1 and ABI4, as the key downstream regulator of TOR signaling to control root growth, meristem activation and seed germination (Kim *et al*. 2016; Barrada *et al*. 2019; Fu *et al*. 2020). Researchers found that TOR has a negative effect on JA signaling pathway and there is a cross-talk between TOR and JA (Song *et al*. 2017). TOR signaling has a significant influence on JA biosynthesis and the associated signal transduction pathways in *cotton* and *Arabidopsis* (Song *et al*. 2017). TOR and SnRK2s work together to regulate the dynamic balance of growth and defense and stress (Jamsheer *et al*. 2019). These results are corresponding to *TOR* cis-acting elements prediction that TOR includes multiple hormone and defense and stress related cis-acing elements (Fig. 2B). *TOR* has cis-acting elements about circadian and some researchers found that metabolite-mediated TOR signaling regulates the

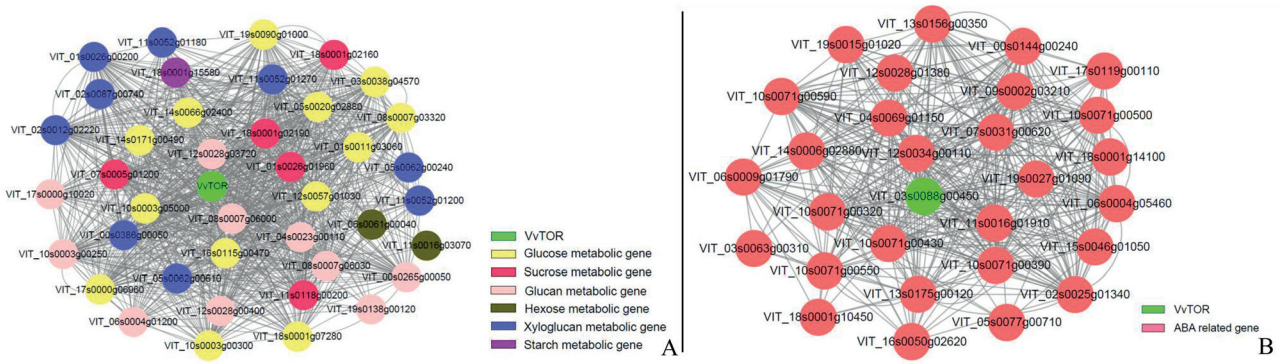

Figure 3. The Co-expression network diagram of *VvTOR* with sugar and ABA related genes. (A) The Co-expression network diagram of *VvTOR* with sugar related genes. (B) The Co-expression network diagram of *VvTOR* with ABA related genes.

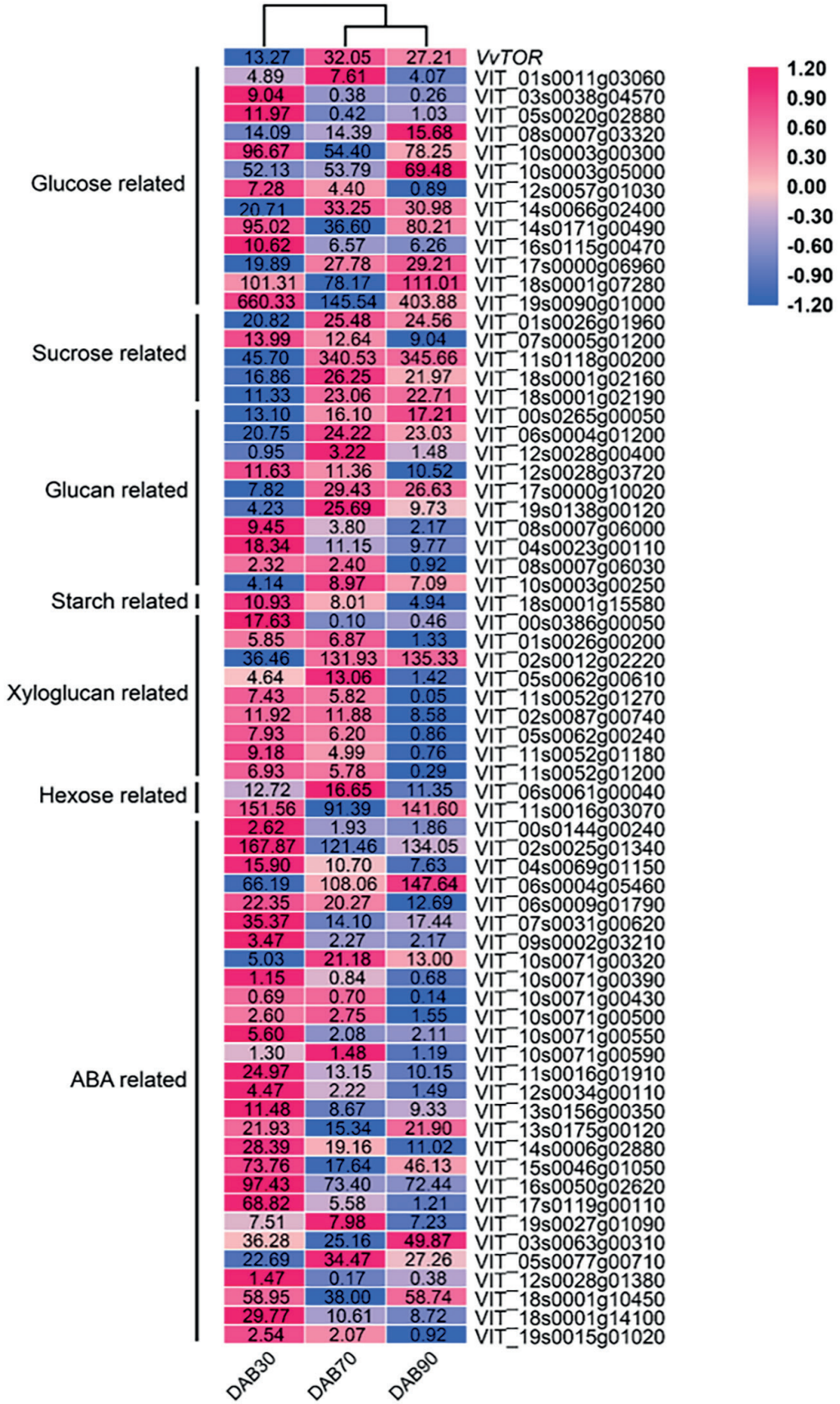

Figure 4. Expression of *VvTOR*, sugar and ABA related genes in three growth and development stage of grape berries. Numerical values in small squares of different colors were FPKM values, representing genes expression data.

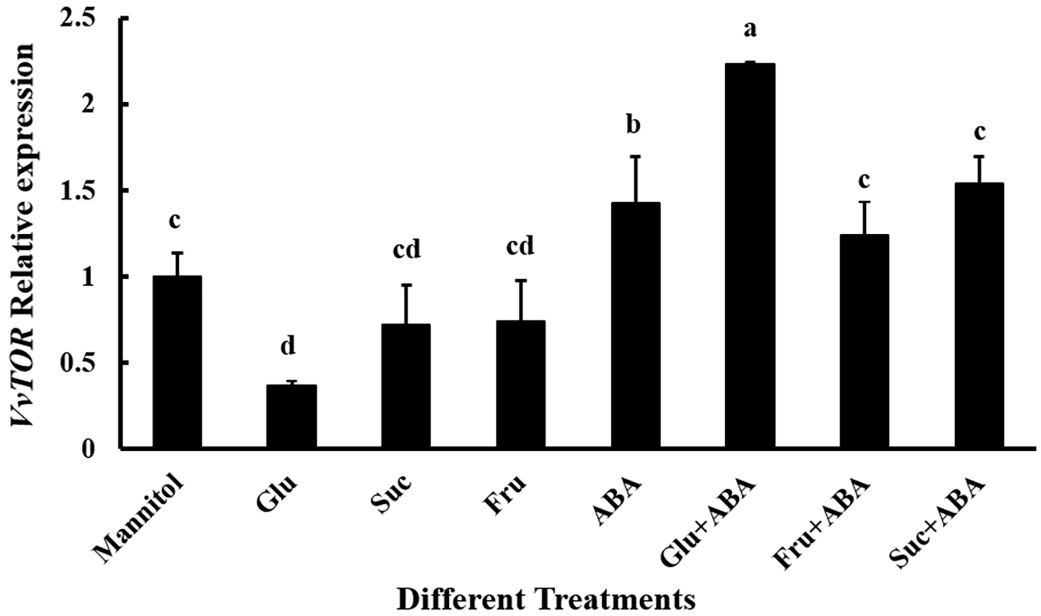

Figure 5. Expression of *VvTOR* in grape suspension cell under mannitol, glucose (Glu), sucrose (Suc), fructose (Fru), abscisic acid (ABA), Glu + ABA, Suc + ABA, Fru + ABA treatments. Data are mean ± SD of three biological replicates. The same letters on the bar are not significantly different by Duncan's test ($p > 0.05$).

circadian clock in *Arabidopsis* and identify TOR kinase as an essential energy sensor to coordinate circadian clock and plant growth (Zhang *et al*. 2019). As for the cis-acting element of meristem expression related (Fig. 2B), the glucose-TOR-E2Fa/b signal network promotes root growth by improving cell division activity in the root meristem (Xiong *et al*. 2013). Some researchers found that cold treatment compromises enhanced anthocyanin accumulation in the inducible *tor-es* mutant under normal temperature, which indicated that TOR may be a negative regulator in cold conditions (Wang *et al*. 2017). This is corresponding to the low temperature responsive cis-acting element (Fig. 2B).

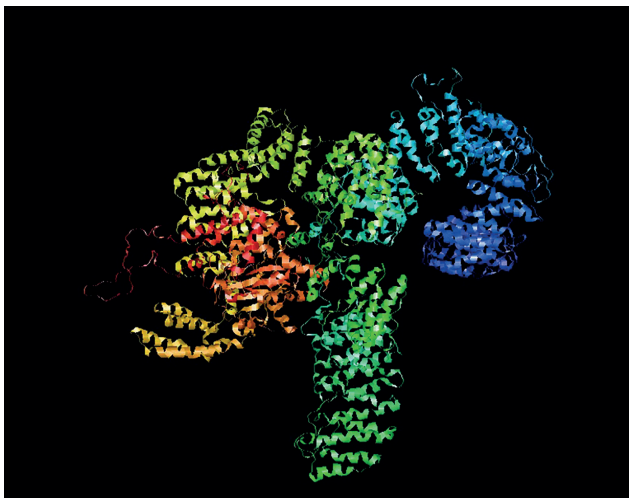

Figure 6. Prediction of the tertiary structure of VvTOR Protein. The quality of the model is indicated by colors from orange (poor quality) to blue (high quality).

Transcriptome sequencing reveals that the early stage of grapevine berries development has a big difference with the middle and later stage (Fig. 4). *VvTOR* expressive levels are high in DAB70 and DAB90, which indicates that *VvTOR* participated in color change and maturity period of grape (Fig. 4). ABA related genes expression levels are higher in DAB30, which suggests more ABA related genes take part in the growth period of grape berries (Fig. 4). The majority of sugar related genes have higher expression levels in DAB70, like sucrose, glucan, xyloglucan, hexose related genes, indicating that these sugar genes may participate in color change of grape (Fig. 4). Sucrose may participate in the maturity period of grapevine because the sucrose related genes have higher expression levels in DAB90 too (Fig. 4). Similarly, xyloglucan related genes expression levels are high in DAB30, indicating that they take part in the growth period of grape berries too (Fig. 4). Glucose related genes had expression in all three stages of grape berries, which may imply that glucose related genes are full participation in the growth and development of grapevine berries (Fig. 4).

VvTOR did not show the presence of signal peptides, suggesting that it was not a secreted protein (Fig. S3). This finding was consistent with its location and function as outer membrane protein without transmembrane structure (Fig. S2). *VvTOR* protein has an amount of alpha helix, which is corresponding to the result of protein secondary structure prediction (Fig. 6) (Guex *et al*. 2009; Bertoni *et al*. 2017; Bienert *et al*. 2017; Waterhouse *et al*. 2018). As a critical protein in the life of grapevine, *VvTOR* protein still has many functions which need to be found and researched, including regulating life-span and responding to light,

auxin and nutrition etc. (Ren *et al*. 2012; Li *et al*. 2017; Schepetilnikov *et al*. 2017). Lately, 63 novel TOR-regulated proteins that have been previously linked to TOR signaling network were discovered(Van Leene *et al*. 2019). Therefore, an in-depth study needs to be done to reveal the important functions of *VvTOR*.

Together, the in silico analysis presented the evolutionary relationship, gene structure, cis-acting elements of *TOR*. The transcriptomic analysis showed the relationship of *VvTOR*, sugar and ABA related genes in the different periods of grapevine berries. Meanwhile, the expression of *VvTOR* in grape suspension cells based on different kinds of sugar and ABA indicated that *VvTOR* had responses to sugar and ABA. These results imply that the potential functions of *VvTOR* in the growth and development of grapevine berries, sugar metabolism and ABA signal pathway. *VvTOR* is hydrophilic and outer membrane protein without transmembrane structure. Consequently, we expect that these in silico analysis are valuable for improving grapevine berries sugar content by regulating *VvTOR* gene and able to offer some viewpoints for exploring the mechanism of *VvTOR* metabolism network.

Acknowledgements

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