Versatile physiological functions of the Nudix Hydrolase family in berry development and stress response in grapevine

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Abstract

Background

Nudix hydrolases are widely distributed across all classes of organisms and provide the potential capacity to hydrolyze a wide range of organic pyrophosphates. Although Nudix hydrolases are involved in plants detoxification processes in response to abiotic and biotic stresses, the biological functions of Nudix hydrolases remain largely unclear in grapevine.

Results

A total of 25 putative grapevine Nudix hydrolases (VvNUDXs) were identified by bioinformatics analysis and classified into eight subfamilies based to their preferred substrates. Both tandem and segmental duplications were responsible for the evolution and expansion of NUDX gene family in grapevine. To investigate into their regulatory roles of VvNUDX genes during growth and development as well as in response to abiotic and biotic stress in grapevine, the expression patterns were revealed in publicly available microarray data. The spatial and temporal expression patterns of VvNUDX genes indicated that these genes might play important roles in multiple developmental processes. Transcriptome and qRT-PCR analysis exhibited that ten VvNUDX genes were specifically expressed in grapevine berries, suggesting the potential roles in grapevine berry development. Expression and phylogenetic analysis demonstrated that VvNUDX1 and VvNUDX3 might be involved in terpenoid biosynthesis in grapevine. Futhermore, most VvNUDX genes toward the ADP-ribose/NADH were different patterns in response to various abiotic and biotic stresses, such as salinity and drought, as well as different types of biotic treatments, such as Erysiphe necator , Bois Noir phytoplasma and leaf-roll-associated virus-3 (GLRaV-3).

Conclusions

These results showed that VvNUDX were associated with plant detoxification processes in response to abiotic and biotic stresses, and regulate disease immunity and resistance pathways. The present informations may provide good opportunities to explore the physiological functions of VvNUDX genes in berry development and stress response networks in grapevine.

Background

The nucleoside diphosphate-linked moiety X (Nudix) hydrolases (NUDX) are a diverse superfamily of pyrophosphohydrolase that are ubiquitous in all classes of life, including archaea, eukaryotes and prokaryotes [1, 2]. The members of Nudix hydrolases are normally characterized by a well-conserved Nudix motif, GX5EX7REUXEEXGU, where U is a bulky hydrophobic residue such as Ile, Leu or Val, and X depicts any residue [1, 3]. Nudix hydrolases catalyze the hydrolysis of nucleoside diphosphate-X (NDP-X) to nucleoside monophosphate (NMP) and phosphate-X (P-X). These NUDX proteins carry out a wide variety of functions to perceive and modulate their substrates levels such as nucleotide diphosphates, nucleotide sugars, deoxyribonucleoside triphosphate (dNTPs), and capped mRNAs [4–6]. Some non-nucleotide substrates have also been identified in the presence of NUDXs, and other relevant substrates may also exist [7]. These compounds are important metabolic intermediates, signaling molecules, and/or coenzymes, as well as potentially toxic compounds. Therefore, NUDXs are originally predicted to function in housecleaning to eliminate excessive toxic metabolites and maintain normal cellular homeostasis [7–10].

Increasing evidences suggest that NUDX proteins play important regulatory roles in diverse physiological and biochemical processes, such as metabolic regulation, plant immunity and stress responses [6–8]. The first plant NUDX protein was identitied from *Lupinus angustifolius* as a diadenosine tetraphosphate (Ap_4A) hydrolase [11]. In *Arabidopsis,* 28 NUDXs were identified and further classified into four subfamilies according to phylogenetic analysis [7]. Most NUDXs members in *Arabidopsis* (AtNUDXs) are localized in the cytosol (AtNUDX1 to 11 and 25) and there are also organelle-type AtNUDXs which distribute in the mitochondria (AtNUDX15) and chloroplasts (AtNUDX14, 19, 23, 26 and 27) [4]. The enzyme activities of all AtNUDXs toward different substrates have been identitied by recombinant proteins in vitro [4,9, 12, 13]. Six AtNUDX gene (AtNUDX2, 6, 7, 10, 14, and 19) exhibit pyrophosphohydrolase activity toward both ADP-rib and NADH [7]. Overexpression of AtNUDX2 increases tolerance to oxidative stress by maintenance of NAD^+ and ATP levels by nucleotide recycling from free ADP-ribose molecules under oxidative stress conditions [14].

Both AtNUDX6 and AtNUDX7 genes are involved in the regulation of stress responses and plant defense. AtNUDX6 directly participates in plant immune response as a positive regulator of NPR1 (nonexpressor of pathogenesis-related genes 1) -dependent SA signaling pathways by modulating NADH levels [15]. Knockout of AtNUDX6 and its overexpression plants show decreased and increased expression of several SA-induced, NPR1-dependent genes and TRX-h5 involved in SA-induced NPR1 activation, respectively. Additionally, the level of NADH in *AtNUDX6* knockout mutants and its overexpression plants was accumulated and reduced, respectively [15]. Contrarily, *AtNUDX7* negatively regulates plant defense response, and its loss-of-function mutation displays constitutive expression of defense-related genes and results in

enhanced resistance against bacteria pathogen *Pseudomonas syringae* [16, 17]. It has been demonstrated conclusively that AtNUDT7 negatively regulates ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1) signaling pathway, which is one of the pivotal components in plant immunity [18]. AtNUDX7 also plays an indispensable role in seed germination, growth, and development by modulating the NAD: NADH homeostasis [19]. Moreover, *AtNUDX8* play a positive regulator in plant immune responses against pathogen attack [20]. Overexpression of AtNUDX14 in chloroplasts shows conspicuously lower levels of both ADP-glucose and starch, suggesting that AtNUDX14 catalyze the hydrolysis of ADP-glucose linked to starch biosynthesis in chloroplasts [12]. Intriguingly, some new results show that plant NUDX members participates in a terpene synthase-independent pathway for monoterpene biosynthesis [21–23]. Monoterpenes reach up to 70% percent of the scent content in some roses (Rosa x hybrida) cultivars. The cytoplasm protein RhNUDX1, which belongs to the Nudix hydrolase family, catalyses dephosphorylation of geranyl diphosphate (GPP) to geranyl phosphate (GP) and responsible for the formation of geraniol and other geraniol-derived monoterpenes that contribute to fragrance in roses [21, 22].

Grapevine is one of the most widely cultivated and commonly consumed fruit crops with high economic and nutritious values [24, 25]. Although a number of NUDX family members have been characterized in Arabidopsis [4,7], Chrysanthemum [26], barley [27] and Brachypodium [28], no comprehensive study of NUDX genes in grapevine (VvNUDX) has been reported so far. Due to the important roles of NUDX during plant growth and development and the release of the grapevine genome [29], a comprehensive analysis were performed to investigate the putative functions of NUDX genes in grapevine. Here, 25 non-redundant VvNUDX genes were identified in grapevine and a systematic analysis including chromosome location, phylogenetic relationships, gene structure, conserved motif and cis-acting elements were performed. We further analyzed the expression of VvNUDX genes in diverse tissues, different stages of fruit development and ripening, as well as in response to hormones and stress treatment. This study provides reliable investigation of the VvNUDX genes and facilitates further functional characterization of VvNUDX members in grapevine.

Methods

Identification of *VvNUDX* genes in the grapevine genome

Two different methods were employed to identify and annotate *VvNUDX* genes in grapevine genome. Firstly, the hidden Markov model (HMM) profiles of the Nudix domain (PF00293) was employed to screen the grapevine genome database (http://genomes.cribi.unipd.it/grape/). Then, all 28 *Arabidopsis NUDX* members (AtNUDX1–27 and AtDCP2), which were downloaded from the TAIR database (http://www.arabidopsis.org), were used to search the local grapevine genome database by BLASTP program with the E value cutoff set at 1e−5. Subsequently, the potential *VvNUDX* genes were further verified for the presence of the Nudix domain by screening against the SMART (http://smart.embl-heidelberg.de/) and InterProScan (http://www.ebi.ac.uk/Tools/pfa/iprscan/) database, respectively. The ExPASY online tool (https://web.expasy.org/protparam/) was employed to calculate molecular weights, isoelectric points (pI) and grand average of hydropathicity (GRAVY) of all VvNUDX proteins. The WoLF PSORT (http://www.genscript.com/psort/wolf_ psort.html) was used to predict the subcellular location of all VvNUDX proteins.

Sequence alignment and phylogenetic analysis

Multiple sequence alignments of the 25 VvNUDX proteins were performed by using ClustalW version 1.83. A total of 53 plant NUDX proteins were used to construct the phylogenetic tree, including 25 from grapevine and 28 from *Arabidopsis.* The Neighbor Joining (NJ) tree based on the conserved domain protein sequences was constructed by MEGA 7.0 software with bootstrap analysis (1000 replicates) [30]. Moreover, another NJ tree based on full length protein sequences of VvNUDX was also constructed by MEGA 7.0 software in grapevine for further analysis. The motif logos of the VvNUDX were generated by using online MEME program (http://meme.nbcr.net/meme/cgi-bin/meme.cgi) [31]. Below are the parameters of MEME used: maximum number of motifs, 20; minimum motif width, 6; and maximum motif width, 50.

Chromosomal location, Gene structure, and duplication analysis

All VvNUDX genes were mapped to grapevine chromosomes according to physical positions information at the Grape Genome CRIBI website (http://genomes.cribi.unipd.it/) and the map was drafted using MapInspect software. The exon-intron organisation of VvNUDX genes was generated using the Gene Structure Display Server (http://gsds.cbi.pku.edu.cn) [32] by comparing coding sequences with their corresponding genomic sequences, which were obtained from the grapevine genome. Tandem duplications of *VvNUDX* genes were defined by checking their physical locations on individual grapevine chromosomes and were identified as adjacent paralogous on a grapevine chromosome, with no more than one intervening gene [33]. Synteny blocks between grapevine and *Arabidopsis* genomes as well as within the grapevine genome were determined by Quick MCScanX Wrapper and visualized by Dual Synteny Plotter in TBtools (https://github.com/CJ-Chen/TBtools).

Cis-Element analysis for *VvNUDX* gene

The promoter sequences (1, 500 bp upstream of the initiation code) of all *VvNUDX* genes were retrieved from the grapevine genome website CRIBI (http://genomes.cribi.unipd.it/). PlantCARE online program (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was employed to identify the putative *cis*-acting element [34].

Expression patterns of *VvNUDXs* in various organs and different berry developmental stages

The expression patterns of *VvNUDX* genes were established in a *V. vinifera* cv 'Corvina' (clone48) gene expression atlas of various organs at different developmental stages. Microarray data were derived from the NCBI gene expression omnibus (GEO) datasets under the series entry GSE36128 (http://www.ncbi.nlm.nih.gov/geo/) [35]. The mean value of each gene in all tissues/organs were analyzed and graphically represented using Multi Experiment Viewer (MeV) software [36]. The expression patterns of *VvNUDX* genes in three different berry developmental stages were also downloaded from NCBI GEO database (accession numbers GSE77218), which generated by using RNA-sequencing (RNA-Seq) data [37]. 'Fujiminori' grapevine berries were collected and analysed at three points throughout the entire growing season, including the green fruit expanding (40DAF or DAF40), veraison (65DAF or DAF65), and ripe (90DAF or DAF90) stages. Furthermore, we also observed the expression profiles of *VvNUDX* genes from 10 grapevine varieties at four berry development stages based on RNA-seq data, which downloaded from the NCBI GEO datasets (accession numbers GSE62744 and GSE62745) [38]. The 10 grapevine varieties contained five white varieties (Vermentino, Garganega, Glera, Moscato Bianco, and Passerina) and five red varieties (Sangiovese, Barbera, Negro amaro, Refosco, and Primitivo). Berries were collected at four developmental stages, the pea-sized berry stage at 20d after flowering, the berries beginning to touch stage just prior to veraison (Pre_veraison), the berry-softening stage at the end of veraison (End_veraison), and the fully ripe berry stage at harvest [38].

Expression patterns of *VvNUDXs* in response to different stress conditions

Expression patterns of VvNUDX in response to abiotic and biotic stresses were based on microarray data downloaded from GEO datasets (series matrix accession numbers GSE31594, GSE31677, GSE6404, GSE12842 and GSE31660). The mean of expression value of each gene in all tissues/organs were analyzed and graphically represented using Multi Experiment Viewer (MeV) software [36].

Potted 'Summer Black' (hybrids of *V. vinifera* and *V. labrusca)* grapevine trees were futher used to insight into the expression levels of *VvNUDXs* in response to four different abiotic stresses, including Cu, salt, waterlogging and drought stress. Grapevine RNA-seq data sets were obtained from NCBI GEO database (SRA accession no. SRP070475 and SRP074162) or published supplemental data sets [24, 39–41]. Cu stress was sprayed with 100 μM $CuSO_4$ [24] and salt stress was treated with 0.8% NaCl [40]. The control plantlets were similarly treated with distilled water. Drought stress was carry out by withholding water 20 days [39] and waterlogging stress was performed by submerging the plants to water for 48h [41]. Grapevine plantlets grown in the standard conditions were used as a control. The RPKM (Reads Per Kilobase per Million mapped reads) values were used to represent the gene expression level as previous method [42]. The heatmap of *VvNUDX* genes was exhibited using R software (http://www.bioconductor.org/).

Plant growth condition and qRT-PCR analysis

Four-years-old 'Fujiminori' grapevine trees were selected as the experimental material, which was grown in the standard field conditions at Pingdu Experimental Vineyard, Qingdao Agricultural University. To investigate gene expression patterns of *VvNUDX* genes during berry development and ripening, grapevine berry samples were also collected at three-time points: the green fruit expanding stage (40 DAF), veraison (65 DAF) and ripe/harvest stages (90 DAF) throughout the growing season. Each points included three replicate plants, and for each plant three berry clusters.

Total RNA of grapevine berry samples at there different stages were extracted by using cetyltrimethyl ammonium bromide (CTAB) method [43] and RNA was treated with DNase I (RNase free; TaKaRa Biotechnology, Dalian, China) to eliminate residual contaminating genomic DNA. For real-time quantitative PCR (RT-qPCR), 1.5 μg total RNA were used to synthesize the first-strand cDNA using PrimeScript II 1st strand cDNA Synthesis kit (TaKaRa Biotechnology, Dalian, China) according to manufacturer's instructions. The grapevine housekeeping gene Actin (AB073011) was used as the reference gene to normalize the qPCR data. The $2^{-\Delta\Delta CT}$ method was used to analyse the relative expression level [44]. All the experiments were performed with three biological replicates and all primers sequences were presented in Table S1.

Results

Identification of *VvNUDX* genes in grapevine

To identify a global view of the *VvNUDX* genes in grapevine genome, the hidden Markov model (HMM) profile of the Nudix domain (PF00293) was used to search against the grapevine genome (http://genomes.cribi.unipd.it/grape/). Then, 28 *Arabidopsis* NUDX members (AtNUDX1–27 and AtDCP2) were employed to search the local grapevine genome database by DNAtools software. Subsequently, SMART database and InterProScan database was employed to confirm the presence of the conserved Nudix domain. After removing the redundant sequences, a total of 27 putative *VvNUDX* genes were identified in grapevine. However, two members (VIT_04s0023g00600.t01 and VIT_11s0206g00020.t01) encoding IPP isomerases, which lacked the Nudix motif (GX5EX7REUXEEXGU) and, thus, had no activity as the NUDX enzyme. Therefore, we concluded that grapevine possessed 25 *VvNUDX* genes (Table 1). Thereafter, these *VvNUDX* genes were named from *VvNUDX1* to *VvNUDX25* according to their ortholog to the *Arabidopsis* gene (Table 1).

The detailed physical and chemical characterizations of VvNUDX were analyzed by Protparam tool (Table 1), including gene name, protein length, chromosome location, molecular weight, theoretical isoelectric point, aliphatic index and GRAVY. The length of VvNUDX proteins varied widely from 142 (VvNUDX1) to 785 (VvNUDX3) amino acid residues. VvNUDX1 showed the lowest value of the molecular weight (15.77 kDa), while the highest of the molecular weight (88.72 kDa) was observed in VvNUDX3. The theoretical isoelectric point (pI) ranged from 4.68 (VvNUDX11) to 8.54 (VvNUDX21), and the aliphatic index varied from 64.28 (VvNUDX23) to 96.57 (VvNUDX14). The GRAVY of all VvNUDX proteins was less than zero, indicating that VvNUDX were hydrophilic (Table 1).

Protein sequence and phylogenetic analysis of VvNUDX gene family

Alignment analysis showed that all 25 VvNUDX members in grapevine were conserved in the amino acid sequences and contained 23 amino acid residues, which was consistent with the previous report in *Arabidopsis* (Fig. S1). To investigate the evolutionary relationship and potential function of VvNUDX members, 53 conserved domain sequences of NUDX proteins, including 25 from grapevine and 28 from *Arabidopsis,* were used to construct the phylogenetic tree using the neighbor-joining method. As shown in the phylogenetic tree (Fig. 1), *VvNUDX* and *AtNUDX* members were divided into eight subfamilies according to their preferred substrates as follow: (1) 8-oxo-(d)GTP, VvNUDX1; (2) ADPribose/NAD(P)H, VvNUDX2, 5, 6, 7, 8, 10, and 19; (3) ADP-ribose/ADP-glucose, VvNUDX14; (4) CoA, VvNUDX11 and 15; (5) Ap_nA/ppGpp, VvNUDX12, 13, 23 and 24; (6) thiamin diphosphate, VvNUDX20 and VvNUDX22; (7) FAD, VvNUDX9; (8) mRNA cap, VvNUDX25. In addition, most of the subgroups comprised multiple VvNUDXs and AtNUDXs. These findings indicated that plants have developed and amplified NUDX genes in each subgroup to adapt their physiology. Futhermore, VvNUDXs could be classified into four types according to their predicted subcellular localization: the cytosol (VvNUDX1, 3, 5, 11 and 18), chloroplast (VvNUDX2, 7, 9, 14, 16, 17, 19, 20, 22, 23 and 24), mitochondrion (VvNUDX4 and 13) or nucleus (VvNUDX6, 8, 10, 12, and 25).

Gene structure analysis and conserved motif identification

All 24 out of 25 *VvNUDX* genes were distributed unevenly throughout the 11 out of the 19 chromosomes (Fig. S2) and the remaining VvNUDX2 had not yet been assembled to any chromosome according to the current grapevine genome (Fig. S2). Among them, the chromosomes 1, 8 and 11 had the highest number of *VvNUDX* genes (four), while only one *VvNUDX* gene was localized on chromosome 2, 9, 10, 17 and 19. Three *VvNUDX* genes were located on chromosome 12 and two *VvNUDX* genes were distributed on chromosome 13 and 14, respectively (Fig. S2).

To better insight into the phylogenetic relationships of the *VvNUDX* genes, the full-length amino acid sequences of VvNUDX protein were used to construct a new phylogenetic tree, which divided the VvNUDX proteins into four groups (Fig. 2A). As shown in Fig. 2A, the VvNUDX members recognizing the same substrates were divided into the same subgroups. For example, VvNUDX2, 5, 6, 7, 8 and 10 with activities toward ADPribose/NADH tended to group together. VvNUDX11 and 15, which exhibit pyrophosphohydrolase activities toward CoA, were divided into the same subgroup.

Futhermore, five conserved motifs compositions were identified among VvNUDXs by MEME program. As expected, all the 25 VvNUDXs displayed a highly conserved Nudix domain (motif 1) (Fig. 2B and C). Most of VvNUDXs within same subfamily exhibited similar distribution of conserved motifs, which supported the classification of subgroups and evolutionary relationship. For example, both motif 3 and motif 4 were hit in all VvNUDX proteins (VvNUDX5, 6, 7, 8 and 10) with activities toward ADPribose/NADH, except for VvNUDX2. Similarly, all seven VvNUDX proteins (VvNUDX4, 12, 13, 16, 17, 18 and 21) of the same subgroup were characterized by motif 5 in N-terminal and motif 5 in C-terminal (Fig. 2B). All the motifs logos and their correspondence locations of these domains were shown in Fig. 2C. Additionally, to further understand the diversification of VvNUDXs, the structure and number of exon/intron of VvNUDXs were analyzed using the online GSDS tool. As shown in Fig. S3, the VvNUDX members showed a variable number of exons, ranging from 2 (VvNUDX1), 3 (VvNUDX18) to 21 (VvNUDX3).

We found that there were four genes, including 5, 7, 8 and 9 exons, respectively. Three genes contained 4 exons and the remaining three genes contained 6 exons (Fig. S3). This phenomenon indicated that the NUDX gene family had undergone both exon gain and loss during evolution, which might be able to further explain the functional differences of closely related NUDX homologous genes.

Tandem duplication and synteny analysis of VvNUDX genes

Tandem and segmental duplications have been suggested to be responsible for gene family evolution and expansion in plants [45]. To clarify the expansion mechanism of *VvNUDX* gene family, potential gene duplication events were investigated in grapevine genome. Two tandem duplication clusters (VvNUDX5/VvNUDX6/VvNUDX7 and VvNUDX20/VvNUDX22) in *VvNUDX* gene family were identified on grapevine chromosome 12 and 11, respectively. Then, two pairs of segmental duplications (VvNUDX12/VvNUDX13 and VvNUDX10/VvNUDX16) in *VvNUDX* gene family were identified within the grapevine genome (Fig. 3A), suggesting that some *VvNUDX* genes were probably generated by gene duplication. These result suggested that both tandem and segmental duplication events contributed to the expansion of the *VvNUDX* gene family in grapevine. Furthermore, a large-scale comparative synteny maps between grapevine and *Arabidopsis* were peformed at genome-wide levels with purpose to reveal the evolution and function of *NUDX* genes. A total of evelen pairs of *NUDX* genes were identified between grapevine and Arabidopsis (Fig. 3B; Table S2), indicating that most *VvNUDX* genes had orthologous in *Arabidopsis.*

Promoter *Cis*-regulatory elements analysis of *VvNUDX* genes in grapevine

To further understand the gene function and regulation mechanism of *VvNUDX* genes, the cis-regulatory elements in promoter regions (1,500 bp of genomic DNA sequence upstream of the translation starts site) were analyzed by PlantCARE database. As expected, besides the basic TATA and CAAT boxes, three category cis-elements, including plant growth and development, biotic and abiotic stress responses and phytohormone responses were identified in the promoter regions (Fig. 4; Table S3). The growth and development related cis-elements were identified in the promoter regions, such as meristem expression related elements (CAT-box and CCGTCC-box), cell cycle regulation related element (MSA-like element), flavonoid biosynthetic related element (MBSI), seed-specific regulation related element (RY-element) and zein metabolism regulation related element (O2-site) (Fig. 4; Table S3). Among these cis-acting elements, 13 O2-site motifs were identified in promoter region of 11 *VvNUDX* genes, which comprised the largest portion of the growth and development category.

In the phytohormone responsive category, the ABA responsive element (ABRE), ethylene responsive element (ERE) and salicylic acid responsive element (TCA-element) were found in the promoters of 18, 20 and 14 *VvNUDX* genes, respectively (Fig. 4; Table S3). The auxin responsive element (AuxRR-core, TGA-box and TGA-element) and gibberellin responsive element (GARE-motif, P-box and TATC-box) and MeJA responsive element (CGTCA motif and TGACG motif) were observed in 12, 13 and 12 *VvNUDX* genes, respectively (Fig. 4; Table S3). Plenty of hormone-responsive elements were observed in the promoter region of *VvNUDX* genes, revealing that hormones could play important functions in the regulation of plant growth and development. In stress-related responses elements, ARE, which was the most abundant element and involved in anaerobic induction, was found in 18 *VvNUDX* genes. In addition, some other stresses-related elements, such as TC-rich repeats (stress responses), WUN-motif (wound responsive), LTR (low temperature) and MBS (drought-inducibility) were also observed in the promoter regions of *VvNUDX* genes (Fig. 4; Table S3). Our results suggested that *VvNUDXs* might respond to multiple abiotic stresses and had the potential roles to improve abiotic stress responses.

Tissue-specific expression patterns analysis of VvNUDX genes in grapevine

To further investigate the dynamic gene expression and putative roles of *NUDX* gene family members in grapevine, the overall organic-specific expression patterns of *VvNUDX* were observed in the *V. vinifera* cv. Corvina global gene expression atlas, which consists of 42 various organs/tissues at different developmental stages obtained by microarray analysis (Fig. 5; Table S3). Hierarchical clustering was used to present the relative expression levels of *VvNUDX* genes in different tissues. As shown in Fig. 5, *VvNUDX10, 12, 17* and *23* were constitutively high expressed in nearly all tissues tested, whereas *VvNUDX8,* 11and 20 were expressed at a very low level in all tested tissues (Fig. 5; Table S4).

Only a small number of members within the same group shared a similar expression profile in grapevine organs/tissues during development. For example, *VvNUDX16* and *VvNUDX18* displayed relatively high transcript levels in floral organs, such as pollen, stamen and flowering, suggesting that these two genes might plant important roles in the floral development (Fig. 5; Table S4). Most *VvNUDX* genes showed significant tissue-specific expression profiles, possibley suggesting the functional divergence of *VvNUDX* genes in grapevine organs/tissues during development. For example, *VvNUDX1* were relatively high expression in overwinter tissues, such as winter bud, and also showed gradually decreasing expression during berry pericarp development and ripening, suggesting an involvement in berry development and cold

acclimation (Fig. 5; Table S4). *VvNUDX5* displayed the highest expression level in root, suggesting that *VvNUDX6* might be involved in root development. *VvNUDX6* was only strongly expressed in seed of veraison and mid-ripening stages, implying an involvement in seed development and ripening. *VvNUDX7* were very high in root, senescing leaf and bud swell, which indicated that it might play a role in the development of root, senescing leaf and bud swell. *VvNUDX11* were preferentially expressed at high levels in senescing and mature leaves, and at almost undetectable levels in other tissues (Fig. 5; Table S4). Remarkably, several *VvNUDX* members (*VvNUDX2, 3, 13, 14* and *22)* were highly expressed in berries, indicating that these genes might play important roles in berry development and ripening (Fig. 5; Table S4). These results aroused us to investigate the expression patterns of *VvNUDX* genes during different fruit development and ripening stages.

Expression patterns of *VvNUDX* genes during berry developmental and ripening

In order to investigate the potential benefits of *VvNUDX* genes during berry development and ripening, the transcript expression patterns of 25 *VvNUDX* genes were analyzed in three stages of berry developmental by using the expression profiles from the NCBI Gene Expression Omnibus (GEO) DataSets (GSE77218). The relative expression levels of *VvNUDX* were described by hierarchical clustering. As shown in Figure. 6A, *VvNUDX17* and *VvNUDX23* displayed relatively high transcript levels during the whole ripening process,whereas eight *VvNUDX* genes (*VvNUDX1, 4, 8, 9, 11, 18, 20* and *22)* were almost undetectable during different berry developmental stages (Fig. 6A; Table S5). Six *VvNUDXs (VvNUDX6, 10, 12, 14, 16* and *21)* were down-regulated transcript accumulation patterns, while three *VvNUDXs (VvNUDX2, 5* and 17) showed up-regulated expression patterns. For example, the expression of *VvNUDX6* was gradually decreased from veraison till to ripe stage. Similar to *VvNUDX6,* but to a lesser extent, *VvNUDX10, 12, 14, 16* and *21* showed the highest expression at green fruit stage. *VvNUDX5* and *VvNUDX17* exhibited a significant increase expression during berry development and reached their picks at the ripening stage. Additionally, six *VvNUDXs (VvNUDX2, 7, 13, 15, 24* and 25)exhibited relatively stable expresssion patterns (Fig. 6A; Table S5). Different expression patterns of *VvNUDX* genes implied its potential roles in berry development and ripening.

To validate the expression patterns of *VvNUDX* genes in three various berry developmental stages by RNA-seq data, 16 relatively high expression of *VvNUDX* genes were selected to test theirt ranscript abundance at three berry development stages by RT-qPCR. As was expected, qRT-PCR results were highly consistent with the RNA-Seq data except for *VvNUDX3* and *VvNUDX7* (Fig. 6B). For example, *VvNUDX5* and *VvNUDX17* showed dramatically increased expression during berry developmental and ripening. *VvNUDX10, 12, 14, 16, 21* and *24* also depicted the highest expression at the green fruit stage (Fig. 6B). However, the expression profiles of *VvNUDX3* and *VvNUDX7* did not correspond with RNA-Seq data. *VvNUDX3* were lowly expressed in veraison stage from RNA-Seq data, whereas the qRT-PCR result showed slightly high expression in the veraison and ripe stage. *VvNUDX7* showed gradually increased expression during berry developmental and ripening from qRT-PCR analysis, whereas the RNA-Seq data showed stable expression at three berry developmental stages (Fig. 6B). All these results indicated that *VvNUDX* genes might be involved in grapevine berry development and ripening.

To obtain more detailed function informations of the *VvNUDXs* during berry developmental, we futher analyzed the transcript expression patterns among 10 different grapevine varieties by using RNA-seq from the NCBI GEO DataSets (GSE62744 and GSE62745), which included four different berry developmental stages (the pea-sized berry stage at 20d after flowering, the berries beginning to touch stage just prior to veraison, the berry-softening stage at the end of veraison, and the fully ripe berry stage at harvest [38]. As shown in Figure 7, the expression of *VvNUDX23* remained continuously strong expression throughout grapevine fruit ripening, which was corresponded with the previous RNA-Seq and RT-qPCR data. *VvNUDX12* and *VvNUDX21* were preferentially expressed in pea-sized berry stage and decreased rapidly throughout grapevine fruit ripening, which was consistent with the datas from previous RNA-Seq and qRT-PCR analysis. On the contrary, *VvNUDX3* were higher expression level at Pre_veraison stage and End_veraison stage, which was agreed with the qRT-PCR analysis. Additionally, 15 *VvNUDX* genes showed no or slight expression during berry development among all 10 grapevine varieties. All these results suggested that *VvNUDX* genes might play important roles in grapevine fruit development.

Expression patterns of *VvNUDX* genes under different abiotic and biotic stresses

The expression patterns of *VvNUDX* genes in response to both abiotic and biotic stresses were investigated by using microarray data from several previously published papers. In regards to abiotic stresses, the microarray expression analysis were conducted according to transcriptomic response of *V. vinifera* cv 'Cabernet Sauvignon' leaves to short-term salt, water and cold stress (GSE31594), and long-term water and salt stress (GSE31677). The microarray expression of only a minority of *VvNUDX* genes were identified based on the few *VvNUDXs* sequences (cDNA and ESTs) known at the time (Tables S6 and S7). In general, the expression of *VvNUDX24* was rapid down-regulation at all time points by all the abiotic stress treatments, indicating the potential roles of *VvNUDX* as an important part of abiotic stress response. Under cold treatment, *VvNUDX12* were repressed from the first to eight hours, whereas *VvNUDX17* was strongly induced in response to short-term cold stress (Fig. 8A, Table S6). Futhermore, a relatively large group of *VvNUDX* genes, including *VvNUDX7, 10, 12, 14, 17, 23* and *25,* were induced to different degrees by short-term drought and salt stresses. For example, *VvNUDX17* and *VvNUDX23* was significantly up-

regulated after 24 h, and *VvNUDX10, 12* and *25* was induced at all time points of the drought and salt treatment (Fig. 8A, Table S6). The microarray data presented in Figure 8B also supported the putative involvement of *VvNUDX10, 12, 17* and 13 in response to drought and salt stress together other genes such as *VvNUDX24*. Interestingly, all these *VvNUDX* genes that were induced in short-term drought and salt stress responses after 24 h (Fig. 8A, Table S6) appeared to be more strongly induced at 16 days after treatment (Fig. 8B, Table S7), which was the last point of the long-term stress treatments. *VvNUDX24* was again strong downregulation at 12 and 16 days after long-term treatment (Fig. 8B, Table S7).

To obtain the potential roles of the *VvNUDX* genes in responses to biotic stresses, microarray expression datasets were investigated from three different host-pathogen interaction experiments, including the infection of susceptible *V. vinifera* cv. 'Cabernet sauvignon' and the tolerant *V. aestivalis* cv. 'Norton' with *Erysiphe necator* (Table S8) [46]; the inoculation of *Bois Noir* phytoplasma on the *V. Vinifera* cv. 'Chardonnay' and cv. 'Incrocio Manzoni' (Table S9) [47]; and the inoculation of *V. vinifera* cv. 'Cabernet Sauvignon' with grapevine leaf-roll-associated virus–3 (GLRaV–3) during veraison and ripening stages of berry development (Table S10) [48]. *VvNUDX7* and *VvNUDX10* showed more significant increase in Cabernet sauvignon than Norton after 48 hour by *E. necator* infection (Fig. 9A and B, Table S8), indicating that the response of *VvNUDX* genes appeared to be much stronger in the susceptible variety cv. Cabernet sauvignon than in the resistant variety cv. Norton after *E. necator* infection. The majority of *VvNUDX* genes (*VvNUDX7, 13, 14* and *25)* were induced expression in both susceptible variety *V. Vinifera* cv. Chardonnay and the tolerant variety cv. Incrocio Manzoni by *Bois Noir* infection (Fig. 9C, Table S9). Conversely, *VvNUDX12* and *17* were both repressed in response to phytoplasma infection. Finally, the expression profiles of five VvNUDX genes (*VvNUDX7, 12, 14, 19* and *25)* were induced in both veraison and ripening phases after GLRaV–3 infection (Fig. 9D, Table S10). However, *VvNUDX10, 13, 23* and *24* were slight downregulation and *VvNUDX17* was specifically induced during the ripening phase in response to GLRaV–3 infection (Figure 9D, Table S10).

In order to validate previous microarray data and reveal more detail of *VvNUDX* in response to different abiotic stresses, we investigated the expression profiles of *VvNUDX* genes under different abiotic stresses, including $CuSO_4$, NaCl, waterlogging and drought treatment (Figure 7). Expression analyses in response to abiotic stresses were based on previous RNA-seq datas (drought stress, 20 days, SRP074162, waterlogging stress, 48 h, SRP070475, salt, 48h, see Additional file 2 in Int. J. Mol. Sci. 2018, 19, 4019, copper stress, 24 h, see Table S2 in Sci. Rep. 2015, 5, 17749). In the salt stress, three *VvNUDX* genes (*VvNUDX 5, 12* and *23)* were up-regulated (Figure 10, Table S11), which was consistent the previous expression profiles by microarray data that *VvNUDX12* and *23* was induced by short and long term salt stresses (Figure 8A and B, Table S6 and 7). In the drought stress, ten *VvNUDX* genes showed increased expression levels to different degrees and three *VvNUDXs (VvNUDX4, 17 and 21)* were more or less reduced by drought treatment according to the RNA-seq data (Figure 10, Table S11). Similarly, five detected *VvNUDX* genes (*VvNUDX7, 10, 14, 23* and *25)* from microarray data also displayed increased expression and further confirmed the RNA-seq result under drought stress. Additionally, four (*VvNUDX1, 5, 12* and *15)* and one *VvNUDX (*VvNUDX9) genes were up-regulated and down-regulated to different degrees after Cu treatment, respectively (Figure 10, Table S11). Under waterlogging stress, six *VvNUDX* genes showed different expression patterns (Figure 10, Table S11), of which, three *VvNUDX (VvNUDX1, 14* and *15)* were up-regulated, and the remaining three genes (*VvNUDX2, 9* and *12)* were down-regulated expression.

Discussion

Nudix hydrolases are ubiquitous distributed in all kingdoms of life and show the potential functions to hydrolyze a wide range of organic pyrophosphates. At present, the structural characteristics and physiological functions of plant NUDX gene family have been identified in several plant species, such as Arabidopsis [7], Chrysanthemum [26], barley [27] and Brachypodium [28]. However, no systematic and comprehensive analyses of the NUDX gene family in grapevine, an important model for perennial fruit crops plants, have been perfomed. In our study, 25 non-redundant *VvNUDX* genes were identified and analyzed from grapevine genome. Then, a multi-level analysis of *VvNUDX* genes were peformed by investigating their phylogenetic relationships, protein motifs, gene structure, cis-acting elements, expression patterns in various tissues and developmental stages and under different stress treatments. The genome-wide information of *VvNUDX* genes will not only provide novel insights into the physiological roles, but also help to establish the groundwork for future functional research of these genes during grapevine growth and development.

Evolution of the *VvNUDX* gene family

NUDXs are widely present across all classes of organisms and thousands of open reading frames (ORFs) potentially encoding NUDXs have been identified in over 360 different species by bioinformatics analysis [2, 10,49]. The number of NUDX family member in each species changes from one in *Mycoplasma* sp. to over 50 in eukaryotes [2]. In plants, previous research showed that *Arabidopsis, Oryza sativa, Populus trichocarpa, Solanum lycopersicum* and *Vitis vinifera* possess 32, 33, 53, 32 and 30 *NUDX* genes, respectively [2]. However, the latest study revealed that there were 28 and 20 NUDX genes in *Arabidopsis* and *Oryza sativa,* respectively [7]. These results indicated that the

numbers of *NUDX* genes must be carefully identified in different species due to the relatively low similarities and identities in the amino acid sequences of NUDXs. In grapevine, two genes encoding IPP isomerases have been removed from *NUDX* gene family due to lack of the Nudix motif (GX5EX7REUXEEXGU). Therefore, our results revealed that the number of genes encoding NUDX proteins were 25 in grapevine. The divergences observed in these NUDX numbers from those previous reported by Kraszawska [2] may rely on the existence of putative alternative splicing variants and/or another homologous protein family such as IPP isomerase.

Additionally, the number of NUDX genes was no relationship to total genome size in higher plants. For example, the *Oryza sativa* (466 Mb) had 20 members, grapevine (490 Mb) had 25, while *Arabidopsis thaliana* (125 Mb) had 28 members. The expansion of certain NUDX families may be caused by selective amplification or retention after genome duplication, the members of which may display divergences in subcellular localization and/or tissue-specific expression. Gene duplication events play important roles in genomic rearrangements and expansions [50] and are identified as either tandem duplications, with two or more adjacent genes located on the same chromosome, or segmental duplications, with duplicated genes present on different chromosomes [51]. Our results demonstrated that both tandem and segmental duplications played major roles in the expansion of *VvNUDX* gene family (Fig. 3). The large number of gene duplication events can provide a reference for the *NUDX* gene evolution analysis and functional prediction in grapevine. Futhermore, phylogenetic analysis showed that almost all VvNUDX subgroups contained at least one homolog of *Arabidopsis* NUDXs (Fig. 1), implying that the subgroups of NUDXs were usually conserved in dicotyledonous plants, and appeared prior to the evolutionary branch point of plant species from other organisms. In addition, almost all VvNUDX members in each subgroup were clustered in the monophyletic group (Fig. 1) as previous reports in *Arabidopsis [7*], implying that the *NUDX* genes with the same substrate specificity may be duplicated from a common ancestor by recent segmental duplication events.

Potential roles of *VvNUDX* genes in grapevine berry growth and development

Grape berry development and ripening is a complex dynamic process that involves a series of molecular genetic and metabolic changes [52]. Increasing evidences demonstrate that NUDX genes play important roles in biosynthesis of terpenoids, which are important aroma and flavor compounds in fruits and flowers [21, 23]. For example, *RhNUDX1* promoted formation of the monoterpene geraniol in petals of scented rose cultivars. Compared with petals expressing control GFP, knockout expression of *RhNUDX1* by RNAi had significantly fewer monoterpenes. All these results showed that the expression levels of *RhNUDX1* positively correlated with the production of the monoterpene geraniol, suggesting its important role in scent production in roses [21, 22]. In *Arabidopsis,* both *AtNUDX1* and *AtNUDX3* catalyzed the transformation of isopentenyl diphosphate (IPP), dimethylallyl diphosphate (DMAPP), geranyl diphosphate (GPP) and farnesyl diphosphate (FPP) into the monophosphate products, isopentenyl phosphate (IP), dimethylallyl phosphate(DMAP), geranyl phosphate (GP) and farnesyl phosphate (FP), respectively [23]. Volatilization of monoterpene linalool was increased 148–503% and volatilization of sesquiterpene β-carophyllene increased by 28–60% from *Arabidopsis* flowers in all *nudx1* and *nudx3* T-DNA mutants. On the contrary, the emission of both monoterpenes and sesquiterpenes by transiently overexpressed of either *AtNUDX1* or *AtNUDX3* was decreased compared to leaves infiltrated with Agrobacteria harbouring an empty vector [23]. These terpenoid metabolic profiles in the knockout and overexpression plants indicated that both *AtNUDX1* and *AtNUDX3* regulate the availability of metabolites contributing to both GPP- and FPP-derived terpenoids.

In grapevine, *VvNUDX1,* the closest homolog of *AtNUDX1* and *RhNUDX1* (Fig.1), displayed lower expression levels in almost all tissues (Fig. 5), which was consistent with the expression profile of *AtNUDX1 [*23]. The similar expression patterns implyed that *VvNUDX1* is likely to perform roles similar to *AtNUDX1* in grapevine. Futhermore, *RhNUDX1* was predominantly expressed in petals, and showed gradually increased expression at later stages of flower development when aroma emission reach its maximum [21]. On the contrary, *VvNUDX1* exhibits gradually decreased expression levels during berry development (Fig. 5 and Fig. 6) when monoterpenoids emission reach its maximum. These results further supported for the proposals that the expression of *VvNUDX1* might be negatively correlated with the production of the monoterpenoids in grapevine berry. *VvNUDX3*,which was closely related to *AtNUDX3 (*Fig. 1),showed significantly higher relative expression levels than *VvNUDX1* in all tested grapevine tissues (Fig. 5). This result was in good agreement with the previous expression patterns with *AtNUDX3* messenger RNA at significantly higher levels than those of *AtNUDX1 [*23]. These expression similarities indicated that *VvNUDX3* was likely to play similar roles to *AtNUDX3* in grapevine terpenoids biosynthesis. Additionally,RNA-seq and qRT-PCR datas showed that *VvNUDX6, VvNUDX10, VvNUDX12, VvNUDX16* and *VvNUDX24* were high transcript expression levels in early fruits in grapevine (Fig. 6), implying that these three *VvNUDX* members were likely to be involved in early grapevine fruit development. On the contrary, *VvNUDX5, VvNUDX7* and *VvNUDX17* showed an increased expression during the ripening of grapevine (Fig. 6), suggesting an involvement of these *VvNUDX* members in berry ripening.

Abiotic and biotic stresses responsive expression of *VvNUDX* in grapevine

Plants are sessile organisms and constantly face challenges by a complex array of abiotic and biotic stresses such as salinity, drought, heavy metal, temperature change and pathogen attack [53]. Plants have evolved sophisticated and remarkable mechanisms to cope with these abiotic and biotic stresses by modulating gene expression [54]. Stresses also generate an excess of reactive oxygen species (ROS) and eventually leads to the oxidative stress [55]. One of the most important molecular mechanism of plant stress response is the elimination of ROS to protect macromolecules and DNA. This scavenging mechanism includes different ROS scavenger enzymes and "house-keeping" enzymes [26]. The family of Nudix hydrolases is one of these "house-keeping" enzyme families and these members are widely distributed among all classes of organisms, such as bacteria, yeast, nematodes, algae, vertebrates, and plants [2,8, 26].

Nudix hydrolases play important roles in plants detoxification processes in response to abiotic and biotic stresses, and are also associated with disease resistance pathways [7]. NADPH is one of the most essential cofactor in cell growth, proliferation and detoxification [56]. Seven *AtNUDX* genes (*AtNUDX2, 6, 7, 10, 14, 19* and *23)* show pyrophosphohydrolase activities toward both ADP-ribose and NAD(P)H and contribute to maintain the energy and redox homeostasis, indicating the importance role of the metabolic regulation of ADP-ribose and NAD(P)H in plant cells. The expression level of *AtNUDX7* was markedly induced by different oxidative stresses, such as paraquat (PQ), ozone, O_2^- and H_2O_2, as well as different types of abiotic treatments, such as salinity, drought and wounding [57–59]. Overexpression and knock-out of the *AtNUDX7* led to increased and decreased tolerance to oxidative stress, respectively, resulting from control NAD^+ levels by supplying ATP via nucleotide recycling from free ADP-Rib molecules [57, 58]. AtNUDX19 could coordinate the regulation of oxidative and hormonal responses by modulating NADPH pool levels and redox homeostasis in chloroplasts [7, 60].

In grapevine, a series of stress-responsive cis-acting elements, such as ARE, WUN-motif, TC-rich, and MBS, frequently occurred in the promoter regions of *VvNUDX* genes (Fig. 4), indicating their potential functions in response to abiotic and biotic stresses. *VvNUDX7* and *VvNUDX10* were closely related to *AtNUDX7* and *AtNUDX10,* respectively (Fig. 1). The phylogenetic analysis implied that these NUDX genes might share similar substrate, which was belonged to the ADP-ribose/NADH subfamily. Both *VvNUDX7* and *VvNUDX10* were induced expression by short and long term drought and salt stresses by microarray and RNA-seq data (Fig. 8 and 10), indicating putative roles of *VvNUDX7* and *VvNUDX10* in grapevine plant stress tolerance. Another *VvNUDX* genes encoding ADP-ribose pyrophosphohydrolase might be *VvNUDX14,* and its expression was also slightly induced by salt and drought stresses based to microarray and RNA-seq data (Fig. 8 and 10). These results indicated that the NUDX members of ADPribose/NAD(P)H subfamily might simultaneously regulate intracellular levels of NAD^+ and ATP via nucleotide recycling from free ADP-ribose molecules in response to stress conditions. Furthermore, *VvNUDX12, VvNUDX23* and *VvNUDX24* were identify to belong to the Ap_nA subfamily, which was not as well characterized as the ADPribose/NAD(P)H subfamily. In yeast and bacteria, heat shock led to a 100-fold increase in the concentration of ApnA level [61]. Both ClNUDX3 and ClNUDX8, which belonged to Ap_nA subfamily, were induced expression by salt, drought, cold, and heat treatment in *Chrysanthemum lavandulifolium* [26]. In our current results, the expression of *VvNUDX24* was suppressed, while *VvNUDX12* and *VvNUDX23* was induced expression by salt and drought based on the microarray and RNA-seq data (Fig. 8 and 10), indicating that they were also involved in the detoxification process under various abiotic stress conditions.

Regarding biotic stresses, *AtNUDX6* and *AtNUDX7* are involved in the modulation of biotic stress responses. *AtNUDX6* was directly participated in the plant immune response as a positive regulator of NPR1-mediated defense [15]. The expression of *AtNUDX7* was induced by avirulent, virulent, and nonhost pathogenic attacks and knock-out of the *AtNUDX7* plants display increased resistance to both virulent and avirulent pathogenic strains [18, 62]. Furthermore, *AtNUDX8* was also participated in SA signaling and positively regulated plant immune responses against pathogen attack [20]. Erysiphe necator, Bois Noir and GLRaV–3 infection, are common biotic stresses in vineyards and negatively impact grapevine growth and development. *VvNUDX7* and *VvNUDX14,* two members of ADPribose/NAD(P)H subfamily, were up-regulated in response to E. necator, Bois Noir and GLRaV–3 infection (Fig. 9), implying that these two *VvNUDX* genes might be involved in the pathogen response pathway. In addition, *VvNUDX1*0 was induced and reduced expression in response to E. necator and GLRaV–3 infection, respectively, suggesting that *VvNUDX10* might have different mechanisms to maintain protection against various biotic signals. All detected *VvNUDX* members of ADPribose/NAD(P)H subfamily were induced by both abiotic and biotic stresses, demonstrating that these genes played important roles in mediating plant defense mechanisms in grapevine and thus deserved further investigation. Currently, the biological function of most *VvNUDX* genes in physiological and developmental processes and plant defence is still unknown and needs to be explicated in grapevine. The present bioinformatic analysis and expression patterns of the *VvNUDX* genes will provide an overall information for selecting candidate genes and facilitate further functional investigation in grapevine.

Conclusions

In the present study, 25 *VvNUDX* genes were bioinformatically characterized from grapevine genome. The *VvNUDX* family genes were divided into eight subfamilies based to their preferred substrates, which were futher supported by high similar exon-intron structures and motif compositions. Gene duplication analysis indicated that both tandem and segmental duplications contributed to the expansion of the

grapevine *NUDX* gene family. *VvNUDX* genes participated in multiple developmental processes as indicated by their spatial and temporal expression patterns. Transcriptome sequencing and qRT-PCR analysis revealed that the *VvNUDX* genes play an important role in fruit developmental stages and might be involved in terpenoid biosynthesis in grapevine. Most *VvNUDX* members, which belonged to the ADP-ribose/NADH subfamily, showed different patterns in response to various abiotic and biotic stresses, and provide good candidate genes for exploring the functions of *VvNUDX* genes in grapevine stress response networks.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

Abbreviations

ABRE: abscisic acid responsive element; DAF: days after flowering; dNTPs: deoxyribonucleoside triphosphate; NDP: nucleoside diphosphate; EDS1: ENHANCED DISEASE SUSCEPTIBILITY1; ERE: ethylene responsive element; GFP: green fluorescent protein; GP: geranyl phosphate; GPP: geranyl diphosphate; HMM: hidden Markov model; GRAVY: grand average of hydropathicity; pI: isoelectric points; NMP: nucleoside monophosphate; NPR1: nonexpressor of pathogenesis-related genes 1; NUDX: nucleoside diphosphate-linked moiety X hydrolases; RPKM: Reads Per Kilobase per Million mapped reads.

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Authors' contributions

XP Leng and GS Liu designed the study and guided the research. ZK Wang and PP Wang was responsible for the main part of data analysis and experimental design. L Guan and M Nasim participated the data analysis. MS Haider performed qRT-PCR. JG Fang provided the experimental materials and manuscript revision. XP Leng contributed to the writing of the manuscript. All the authors have commented, read and approved the final manuscript.

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Table

Table 1. NUDX gene family in grapevine

Gene Name	Accession number	Protein	Chrom	Chr srart	Chr end	MW(Da)	pI	Aliphatic index	GRAVY	Loc
VvNUDX1	VIT_01s0011g02750.t01	142	Chr1	2465257	2465757	15773.85	4.99	80.28	-0.207	cyto: 10, chlo: 2, mito: 2
VvNUDX2	VIT_00s0259g00200.t01	253	Chrun	18507436	18511498	27970.37	7.10	92.41	-0.007	chlo: 11.5, chlo_mito: 7.5
VvNUDX3	VIT_09s0018g01600.t01	785	Chr9	18744653	18788329	88717.44	5.26	92.22	-0.227	cyto: 9, nucl: 3, chlo: 1
VvNUDX4	VIT_14s0006g00300.t01	182	Chr14	13579253	13580372	21142.27	7.64	80.82	-0.528	mito: 6.5, chlo: 5, cyto_mito: 4
VvNUDX5	VIT_12s0057g01110.t01	302	Chr12	9776927	9782095	34095.93	6.62	78.44	-0.380	cysk: 11, cyto: 2
VvNUDX6	VIT_12s0057g01090.t01	370	Chr12	9762881	9766576	41015.09	5.89	82.16	-0.101	nucl: 4, cyto: 3, E.R.: 3, vacu: 2
VvNUDX7	VIT_12s0057g01100.t01	341	Chr12	9771111	9775976	38014.37	8.52	84.34	-0.237	chlo: 11, mito: 3
VvNUDX8	VIT_11s0016g02860.t01	221	Chr11	2308784	2310127	25535.68	5.92	89.95	-0.145	nucl: 7, cyto: 5, mito: 1
VvNUDX9	VIT_08s0007g06540.t01	291	Chr8	20265353	20272464	32008.69	8.72	77.46	-0.187	chlo: 9, mito: 4
VvNUDX10	VIT_19s0015g00550.t01	282	Chr19	8601741	8608554	31929.41	5.26	85.71	-0.249	nucl:6,cyto:4,cysk:3.5
VvNUDX11	VIT_08s0058g00170.t01	284	Chr8	8809248	8810569	31914.51	4.68	88.52	-0.113	cyto: 6, chlo: 4, nucl: 2, cysk: 2
VvNUDX12	VIT_01s0150g00320.t01	222	Chr1	22725177	22728397	25613.21	6.97	70.27	-0.661	nucl: 5, mito: 4, chlo: 3, cyto: 2
VvNUDX13	VIT_14s0068g00870.t01	215	Chr14	24631338	24634655	25051.11	5.06	68.93	-0.739	mito: 5.5, chlo: 5, cyto_mito: 3.5
VvNUDX14	VIT_02s0025g04840.t01	306	Chr2	4365378	4369488	33723.03	7.65	96.57	-0.105	chlo: 7, plas: 2, vacu: 2, nucl: 1
VvNUDX15	VIT_10s0003g00880.t01	520	Chr10	2121091	2127806	58149.11	5.18	87.94	-0.374	plas: 7, chlo: 5, mito: 1
VvNUDX16	VIT_08s0007g05200.t01	182	Chr8	19114404	19118950	20660.44	5.59	75.60	-0.553	chlo: 6, mito: 4, nucl: 2, cyto: 2
VvNUDX17	VIT_01s0011g05670.t01	168	Chr1	5374629	5375670	19468.29	5.38	77.62	-0.501	chlo: 6, cyto: 4, plas: 3
VvNUDX18	VIT_13s0084g00690.t01	196	Chr13	19868745	19872416	22593.50	5.15	69.18	-0.578	cyto: 7, nucl: 2, chlo: 1, mito: 1
VvNUDX19	VIT_11s0016g04950.t01	441	Chr11	4261292	4266786	49227.35	6.68	81.38	-0.230	chlo: 11.5, chlo_mito: 7.5
VvNUDX20	VIT_11s0016g04300.t01	366	Chr11	3591369	3596486	40871.24	7.64	94.84	-0.021	chlo: 4, cyto: 3, mito: 2, vacu: 2
VvNUDX21	VIT_17s0000g02050.t01	213	Chr17	1666578	1668037	24581.39	8.54	76.38	-0.434	chlo: 12, cyto: 1
VvNUDX22	VIT_11s0016g04320.t01	364	Chr11	3606185	3611360	40990.96	8.27	86.70	-0.182	chlo:9.5,chlo_mito:7.33333
VvNUDX23	VIT_01s0011g04950.t01	173	Chr1	4586268	4590991	19686.09	4.91	64.28	-0.641	chlo: 8, cyto: 4, nucl: 1
VvNUDX24	VIT_08s0056g00030.t01	228	Chr8	36089	44131	26036.61	5.53	69.25	-0.496	chlo: 13
VvNUDX25	VIT_13s0019g04310.t01	321	Chr13	5615546	5625464	36271.18	6.02	79.88	-0.344	nucl: 8, chlo: 2, mito: 2, cyto: 1

AA, amino acid residues, Chrom, chromosome; MW, molecular weight; pI, theoretical isoelectric point; GRAVY, grand average of hydropathicity, Loc, subcellular location. The subcellular location results of VvNUDX genes were predicted by WoLF PSORT (https://www.genscript.com/wolf-psort.html). Chlo, chloroplast; Cyto, cytosol; Cysk, cytoskeleton; Mito, mitochondria; Nucl, nucleus; Plas, plasma membrane; Vacu, vacuolar. Testk used for kNN is: 14

Supplementary Files Legend

Figure S1. Alignment of partial amino acid sequences surrounding the Nudix motifs in VvNUDXs.

Figure S2. Chromosomal distribution of VvNUDX genes. Chromosome numbers are provided at the top of each chromosome together with the approximate size.

Figure S3. Gene structure of the VvNUDX family generated from GSDS. The yellow block means the coding sequence (CDS), the blue block means the upstream or downstream of the genes, and the black line indicates the intron. The scale bar indicates the length of the DNA sequences.

Table S1. The primers sequences of VvNUDX genes for qRT-PCR.

Table S2. The segmental duplication events between grapevine and Arabidopsis.

Table S3. Promoter analysis of the grapevine NUDX gene family.

Table S4. Expression profiles of the grapevine VvNUDX genes in different organs, tissues and developmental stages.

Table S5. Expression profiles of the grapevine VvNUDX genes during three fruit developmental stages.

Table S6. Expression profiles of the grapevine VvNUDX genes in response to short-term abiotic stress.

Table S7. Expression profiles of the grapevine VvNUDX genes in response to long-term abiotic stress.

Table S8. Expression profiles of the grapevine VvNUDX genes in response to E. necator infection.

Table S9. Expression profiles of the grapevine VvNUDX genes in response to Bois Noir infection.

Table S10. Expression profiles of the grapevine VvNUDX genes in response to GLRaV–3 infection.

Table S11. Expression profiles of the grapevine VvNUDX genes in response to abiotic stress.

Figures

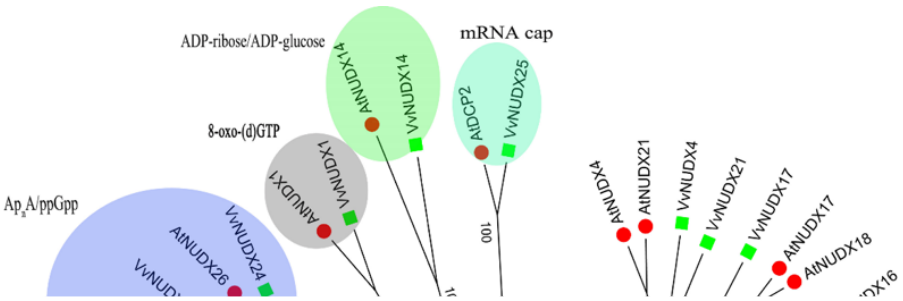

Figure 1

The phylogenetic tree of NUDXs from Arabidopsis and grapevine. The phylogenetic tree was constructed using the neighbor-joining method with1000 bootstrap replicates by MEGA7.0. The preferred substrate is indicated. The scale bar corresponds to the branch length and shows

0.1 amino acid substitutions per site.

Figure 2

Phylogenetic analysis and conserved motifs of NUDX family in grapevine. (A) The full-length amino acid sequences of VvNUDX proteins were constructed a Neighbor-Joining phylogenetic tree with1000 bootstrap replicates by MEGA7.0. (B) Distribution of conserved motifs of VvNUDX proteins. Different motifs were shown by different colors numbered 1 to 5. See legend for detailed color. (C) The conserved protein motifs in the VvNUDX proteins. The x-axis indicated the conserved sequences of the domain. The height of each letter indicated the conservation of each residue across all proteins. The y-axis was a scale of the relative entropy, which reflected the conservation rate of each amino acid.

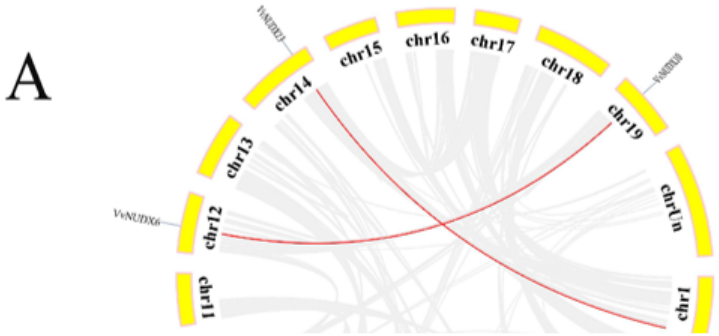

Figure 3

Synteny analysis of the NUDX genes. (A) Synteny analysis of the VvNUDX genes in grapevine. Chromosomes 1-19 were shown with yellow circular form. Red curves denote the details of syntenic regions between VvNUDX genes. (B) Synteny analysis of the NUDX genes between grapevine and Arabidopsis. Gray lines in the background indicated the collinear blocks between grapevine and Arabidopsis genomes, and the red lines highlight the syntenic NUDX gene pairs.

Figure 4

Promoter Cis-regulatory elements analysis of grapevine VvNUDX genes. Based on the functional annotation, the cis-acting elements were classified into three major classes: plant growth and development, phytohormone responsive, or abiotic and biotic stresses-related cis-acting elements. The different colors and numbers of the grid indicated the numbers of different promoter elements in these VvNUDX genes.

Figure 5

Expression profiles of grapevine VvNUDX genes in various tissues and developmental stages. Expression data were normalized based on the mean expression value of each gene in all tissues analysed. Genes were hierarchically clustered based on average Pearson's distance metric and 'average linkage' method. Red and green boxes indicate high and low expression levels, respectively, for each gene. Bud-AB, bud after burst; Bud-B, Bud burst; Bud-W, winter bud; Bud-L, latent bud; Bud-S, bud swell; Flower-F, flowering; Flower-FB, flowering begins; FS, fruit set; Inflorescence-Y, young inflorescence with single flowers separated; Inflorescence-WD, well-developed inflorescence; Leaf-FS, mature leaf; Leaf-S, senescing leaf; Leaf-Y, young leaf; MR, mid-ripening; R, ripening; PFS, post fruit set; Stem-G, green stem; Stem-W, woody stem; V, véraison.

Figure 6

Expression profile of grapevine VvNUDX genes during three fruit developmental stages. A, Hierarchical clustering of the transcript accumulation profiles of 25 VvNUDX genes during three berry developmental stages. B, RT-qPCR transcript analysis of 16 selected VvNUDX genes at three berry developmental stages. 'Fujiminori' grapevine berries were sampled in triplicate at the fruit expanding (40DAF or DAF40), veraison (65DAF or DAF65), and ripe (90DAF or DAF90) stages throughout the growing season.

Figure 7

Expression profiles of the grapevine VvNUDX genes in 10 different grapevine varieties at four berry developmental stages. Berries were sampled at four developmental stages, the pea-sized berry stage at 20d after flowering, the berries beginning to touch stage just prior to veraison (Pre_veraison), the berry-softening stage at the end of veraison (End_veraison), and the fully ripe berry stage at harvest.

Figure 8

Expression patterns of VvNUDX genes in response to abiotic stresses. Microarray analysis of VvNUDX genes in the V. vinifera cv 'Cabernet Sauvignon' were downloaded from the NCBI GEO datasets (GSE31594 and GSE31677) and graphically represented with MeV software. (A) V. vinifera cv 'Cabernet Sauvignon' plants grown in a hydroponic drip system were treated with 120 mM salt, polyethylene glycol (PEG), cold (5 °C) or untreated. Shoots with leaves were collected at 0, 1, 4 and 8 h for all treatments, and at 24 h for all treatments except cold (GEO series GSE31594). (B) Potted V. vinifera cv 'Cabernet Sauvignon' vines in the greenhouse were exposed to a water-deficit stress (WD) by withholding water or a salt stress by watering plants with a saline solution for 16 days. Non-stressed, normally watered plants served as the control for both treatments. Shoot tips were harvested every 4 days (0, 4, 8, 12 and 16 days) (GEO series GSE31677).

Figure 9

Expression patterns of VvNUDX genes in response to biotic stresses. (A and B) V. vinifera cv 'Cabernet sauvignon' (Vv) and V. aestivalis cv 'Norton' plants were grown in an environmental chamber and inoculated with Erysiphe necator conidiospores (PM). Inoculated leaves were harvested at 0, 4, 8, 12, 24 and 48 h after inoculation (GEO series GSE6404). (C) Field-grown plants of V. vinifera cv 'Chardonnay' and 'Incrocio Manzoni' naturally infected with Bois Noir phytoplasma (BN), compared to healthy samples (GEO series GSE12842). (D) V. vinifera cv 'Cabernet Sauvignon' was infected with GLRaV-3 during veraison and the ripening stages of berry development (GEO series GSE31660).

Figure 10

The expression of VvNUDX genes under different abiotic stresses. Hierarchical cluster displaying the differentially expressed VvNUDX genes under Cu, drought, salt and waterlogging treatments. Data were obtained by RNA Sequencing and were expressed as Reads Per Kilobase of exon model per Million mapped reads (RPKM). The differentially expressed data were log2 transformed with R software. Blocks with green colors indicate decreased and red ones indicate increased transcription levels.

Supplementary Files

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