Involvement of Ubiquitin-Conjugating Enzyme (E2 Gene Family) in Ripening Process and Response to Cold and Heat Stress of Vitis vinifera

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Ubiquitin-conjugating (UBC) E2 enzyme plays crucial roles in plant growth and development. Limited information can describe the function of UBC enzyme E2 in grapes. A total of 43 UBC enzyme E2 genes with conserved UBC domain were identified in grapes. These genes were divided into five groups based on phylogenetic tree with tomatoes. Sequence analyses indicated that VvUBCs in the same group possessed similar gene structures and conserved motifs. Gene distribution in chromosomes was uneven, and gene duplication existed in 36 VvUBCs. Transcriptome and qRT-PCR analysis indicated that most VvUBCs are involved in ripening and post-harvest stage, and feature functional roles in grape organs. According to the transcriptome and qRT-PCR results, seven and six VvUBCs in grape responded to cold and heat stress, respectively, whereas no remarkable VvUBCs change was noted under salt or water-deficit stress. This study provides new insights to physiological and developmental roles of these enzymes and regulation mechanism of E2 genes in grapes.

Ubiquitination is an important type of post-translational modification of proteins among all eukaryotes. This important process regulates a wide range of biological processes1, including intracellular translocation of proteins, chromosomal organization, DNA repair, cell cycle control, and apoptosis2-4.

Ubiquitin covalently binds with target proteins, causing a series of enzyme catalytic effects. This process requires coordination of three types of enzymes, namely, ubiquitin-activating enzyme (E1), ubiquitin-conjugating (UBC) enzyme (E2), and ubiquitin-ligase enzyme (E3)5. Ubiquitin is activated in an ATP-dependent manner linked with E1; E2 accepts ubiquitin from E1, passes it to active-site cysteine, and then transfers ubiquitin to a targeted protein aided by E3. Additional ubiquitin can be further ligated to initial ubiquitin molecule through sequential ubiquitination cycles, ultimately forming a poly-ubiquitin chain; finally, targeted proteins are modified6. Then, substrates can be degraded to generate other biological effects. E2 plays a crucial role in ubiquitination and is responsible for attachment of ubiquitin to targeted proteins7. E2 protein contains a conserved catalytic domain, called the UBC domain, spanning 140–200 amino acids in length. Various studies indicated that UBC domain mediates the interaction between E2 and E38-10. A special interaction occurs between UBC domain in E2 and RING domain in E311.

E2 genes exist as a multi-gene family and are involved in many plant physiological activities. A total of 14, 50, 41, 39, and 75 E2 genes were identified in Saccharomyces cerevisiae12, humans13, Arabidopsis14, rice15, and maize16, respectively. A number of E2 genes are involved in environmental stresses. For example, VrUBC1 of mung bean responded to osmotic17 stress, and E2 genes in soybean and peanut reacted to drought and salt stress in transgenic Arabidopsis18-20. Recently, researchers discovered that fruit-ripening regulator (RIN) can directly bind to the...
promoter of E2 genes in tomato, pigmentation of fruit was altered at orange ripening by silencing of E2 genes. E2 genes are also involved in plant disease resistance through positive plant immune regulation. Some researchers observed association of E2 genes with cryogenic autolysis in Volvariella volvacea. GhUNC1/2 is involved in auxin-associated effects and is related to degradation of target proteins, delaying senescence in cotton.

Grapes (Vitis vinifera) are one of the most important fruit species in the world. Genome sequence of this fruit was released in 2007, it provides foundation for ongoing studies at the genome level. At present, limited information can describe the role of E2 enzyme in grapes. For example, 45 E2 genes family members were identified in 8× coverage assembly of Vitis vinifera PN40024 genome. E2-21 is down-regulated at veraison stage in Cabernet Sauvignon (Vitis vinifera). Until now, no systematical analysis has been performed on E2 genes family to identify their expression during grapevine development and response during abiotic stress.

The following are objectives of the present study: to identify and clarify members of E2 genes family from the 12× coverage assembly grapevine genome, to characterize their expression pattern during grapevine development and berry ripening, and to explore their functions in abiotic stress. Understanding functions of E2 enzymes bears significance in analyzing regulation mechanism of enzymes in grapevines.

Results
Identification of Vitis vinifera UBC enzyme E2 proteins. In this study, 43 unique UBC enzyme members were identified using Hidden Markov Model (HMM) and BLAST search methods (Table 1). All these genes contained the UBC domain. A phylogenetic tree was constructed, with 43 VvUBC members in grapes and 52 SlUBC members in tomatoes. VvUBCs showed the relationship between grapes and tomatoes on the phylogenetic tree.

Phylogenetic analysis of VvUBC family. Phylogenetic analysis showed that 43 VvUBC members can be classified into five groups (Fig. 1 and Table 1). Groups I to V (Table 1) contained 15, 8, 6, 11, and 3 members, respectively. Compared with grapes, SlUBC members in tomatoes were classified into six groups. Group I, II, and IV each included 12 members. Group III and V contained 9 and 6 members, respectively. SlUBC14 existed in Group VI alone (Fig. 1).

Conserved domain analysis. UBC enzyme E2 gene family possesses a highly conserved UBC domain. Similar to E2 of human, VvUBC members can be divided into four classes according to existence of additional extensions to UBC domain (Fig. 2). In the present study, 50 amino acid residues (or less than 50 amino acid but performing other structural domain) beside UBC domain were regarded as additional extension. Most VvUBCs (28 members) possess a single UBC domain and are categorized as Class I. Class II (three members) features an N-terminal extension, Class III (six members) presents a C-terminal extension, and Class IV (six members) exhibits both extensions (see Supplemental Fig. S1). Interestingly, two VvUBCs contain other domains except for UBC. VvUBC27 contains a ubiquitin-associated domain (UBA) at C-terminal, and VvUBC12 contains X8 domain at N-terminal.

Conserved motifs, gene structure, and promoter analysis of VvUBCs. Ten motifs were identified to illustrate VvUBC protein structure using MEME program and further annotated by InterPro Scan. Eight of 10 motifs (except motif 5 and motif 7) were localized within the UBC/RWD (RING finger-containing proteins, WD-repeat-containing proteins, and yeast DEAD or DEXD like helicases) domain, which contained an alpha-beta(4)-alpha(3) core fold, and was found in E2 and related proteins. RING finger and WD repeat-containing proteins, all VvUBCs contained at least two of them. VvUBC proteins contained 2–6 motifs, and length of motifs ranged from 11–50 amino acids (see Supplemental Fig. S1). Motifs 1 and 5 existed in almost all 43 VvUBCs except for five VvUBCs in Group I; by contrast, motifs 3, 2, and 4 existed in 39, 30, and 25 VvUBCs, respectively. The remaining motifs were detected in less than half of VvUBCs. Motifs 6 and 8 only existed in five VvUBCs in Group IV. Motifs 9 and 10 only existed in three VvUBCs in Group I. Group II and Group V respectively featured the same motifs except for VvUBC12.

Figure S3 shows gene structure of VvUBC genes. All VvUBC genes contained at least one untranslated region (UTR) in their 3′ or 3′ terminal and 3–11 exons. VvUBCs presented varying gene lengths ranging from 1267 bp (VvUBC11) to 23957 bp (VvUBC44). Length of coding sequence (CDS) averagely accounted for 10.45% of the whole gene length. This length did not relate to gene length.

Locations of promoter region compared to transcriptional initiation site range from –15000 bp (VvUBC15) to –115 bp (VvUBC38). Tween-three VvUBCs are located in the positive strand, whereas 20 VvUBCs are in the negative strand (Fig. S4).

Chromosome localization and gene duplication analysis of VvUBCs. A total of 43 VvUBCs were distributed in all chromosomes except for chromosome 10, and most genes were close to chromosome terminal (Fig. S5). Chromosomes 6 and 8 contained the most VvUBCs (5 members), and other chromosomes contained 1–3 VvUBCs.

According to the whole genome duplication (WGD) related gene duplication analysis, WGD of VvUBCs occurred during grape genome evolution (Fig. 4), and a group of 36 VvUBCs were involved in 71 WGD events. For example, VvUBC26 located in Chromosomes 1 and VvUBC4 in Chromosome 14 are relative genes. These WGD events accounted for 83.72% (36 of 43) of VvUBCs gene expansion.

Temporal and spatial expression patterns of VvUBCs. A total of 42 VvUBCs (without VvUBC5b) were identified by transcriptome analysis (GSE36128) of 54 organs in Corvina (Vitis vinifera) (Fig. S6). Expression of numerous VvUBCs showed significant changes during grapevine development. VvUBC12 showed decreasing tendency in all organs (Fig. S6). During berry ripening, VvUBC3 increased in three berry tissues (berry...
were expressed in berries. Most genes showed increasing or decreasing expression levels during ripening. Six
similar expression patterns of VvUBCs were expressed specially in different organs. This analysis was performed in
four berry developmental stages (pea size, berry tough, soft, and harvest) (Fig. 5).

| Gene locus ID | Protein length(aa) | Group Chr Start End | NCBI Accession | Additional features |
|---------------|-------------------|---------------------|----------------|-------------------|
| GSVIVT01026953001 | 184 V 15 19076770 19083025 | VvUBC1a | C-terminal extension |
| GSVIVT01019387001 | 183 V 2 236398 241211 | VvUBC1b | C-terminal extension |
| GSVIVT010240005001 | 1099 IV 3 1786769 1794856 | VvUBC3 | N&G-terminal extension |
| GSVIVT01011359001 | 119 III 14 28936555 28941655 | VvUBC4 | N&G-terminal extension |
| GSVIVT01005206001 | 944 IV Un 19190912 19196143 | VvUBCAa | N&G-terminal extension |
| GSVIVT01005576001 | 271 IV Un 40650232 40651612 | VvUBCB | C-terminal extension |
| GSVIVT01009784001 | 161 I 18 11241271 11245493 | VvUBCC | C-terminal extension |
| GSVIVT01009665001 | 197 IV 18 10233988 10235820 | VvUBCC8 | C-terminal extension |
| GSVIVT01008045001 | 152 III 17 6349780 6356685 | VvUBC9 | N&G-terminal extension |
| GSVIVT01022074001 | 162 III 7 16377832 16385165 | VvUBCA10 | C-terminal extension |
| GSVIVT01024207001 | 148 II 3 1661190 1662457 | VvUBCA11 | C-terminal extension |
| GSVIVT01025431001 | 528 II 6 847758 858488 | VvUBCA12 | N-terminal extension |
| GSVIVT01022467001 | 119 III 8 3378128 3385686 | VvUBCA15 | C-terminal extension |
| GSVIVT01016663001 | 305 I 9 206133 216225 | VvUBCA16 | N&G-terminal extension |
| GSVIVT01016569001 | 168 IV 13 2698670 2704723 | VvUBCA17 | C-terminal extension |
| GSVIVT01018860001 | 161 I 4 19153005 19160933 | VvUBCA19 | C-terminal extension |
| GSVIVT01024546001 | 148 II 6 8975439 8978143 | VvUBCA24 | N&G-terminal extension |
| GSVIVT01011671001 | 159 I 1 5344900 5350070 | VvUBCA25a | C-terminal extension |
| GSVIVT01033925001 | 146 I 8 16675006 16681479 | VvUBCA25b | C-terminal extension |
| GSVIVT01034603001 | 146 I 6 21209023 21218935 | VvUBCA25c | C-terminal extension |
| GSVIVT01016300001 | 190 I 13 5369865 5375890 | VvUBCA25d | N-terminal extension |
| GSVIVT01020556001 | 191 III 1 11008741 11013679 | VvUBCA26 | N-terminal extension |
| GSVIVT01008615001 | 150 I 17 394286 397861 | VvUBCA27 | C-terminal extension |
| GSVIVT01009448001 | 160 III 18 8645118 8649528 | VvUBCA29 | C-terminal extension |
| GSVIVT01015392001 | 188 I 11 3245946 3247764 | VvUBCA30 | C-terminal extension |
| GSVIVT01020551001 | 472 IV 12 4458311 4466998 | VvUBCA31 | N&G-terminal extension |
| GSVIVT01020701001 | 153 I 12 3005962 3011245 | VvUBCA32 | C-terminal extension |
| GSVIVT01014758001 | 148 II 19 9349179 9350972 | VvUBCA33 | C-terminal extension |
| GSVIVT01019484001 | 148 II 2 1020201 1030408 | VvUBCA34 | C-terminal extension |
| GSVIVT01025872001 | 148 II 8 10943294 10948994 | VvUBCA36 | C-terminal extension |
| GSVIVT01014343001 | 497 IV 19 2820615 2830331 | VvUBCA38 | N&G-terminal extension |
| GSVIVT01014215001 | 153 I 19 1528687 1547441 | VvUBCA39 | C-terminal extension |
| GSVIVT01008729001 | 148 II 16 19346927 19354509 | VvUBCA40 | C-terminal extension |
| GSVIVT01025833001 | 311 I 8 11398555 11422512 | VvUBCA44 | N&G-terminal extension |
| GSVIVT01031547001 | 177 I 6 17270831 17272824 | VvUBCA45 | C-terminal extension |
| GSVIVT01003565001 | 157 I 4 3045208 3049485 | VvUBCA46 | C-terminal extension |
| GSVIVT01007794001 | 184 V 17 8952004 8971372 | VvUBCA47 | C-terminal extension |
| GSVIVT01031919001 | 183 IV 3 5404938 5409770 | VvUBCA51 | C-terminal extension |
| GSVIVT01035008001 | 297 I 5 854146 857902 | VvUBCA52 | C-terminal extension |

Table 1. Information of Vitis vinifera ubiquitin-conjugating enzymes E2 gene family identified in this study. –represents no additional features.

pericarp, berry flesh, and berry skin), whereas VvUBC34 decreased, VvUBC7 was up-regulated first and then
down-regulated. In post-harvest withering stage, VvUBC3 was rapidly up-regulated in three berry tissues (berry
pericarp, berry flesh, and berry skin) and reached the highest level in post-harvest withering-III stage, whereas
VvUBC7/29/34 were down-regulated significantly. Several VvUBCs were expressed specially in different organs.
For example, VvUBC11 showed low expression level in berry but is highly expressed in leaves, especially in senes-
cent leaves. VvUBC45 featured higher expression level in winter buds than other organs.

To characterize expression pattern of VvUBCs in different genotypes, a transcriptome analysis in five varieties
(Sangiovese, Barbera, Negroamaro, Refosco, and Primitivo) was performed using published data (GSE62744).
This analysis was performed in four berry developmental stages (pea size, berry tough, soft, and harvest) (Fig. 5).
Similar expression patterns of VvUBCs were observed in different varieties. Thirty-seven out of 43 VvUBC genes
were expressed in berries. Most genes showed increasing or decreasing expression levels during ripening. Six
VvUBCs (VvUBC4/11/12/20/34/44) were remarkably down-regulated in the last two stages of ripening (soft and harvest). VvUBC21 was up-regulated at berry tough stage then down-regulated at soft stage. VvUBC45 was significantly down-regulated after pea size stage. Interestingly, VvUBC51 showed higher expression levels in Sangiovese than other four varieties. The other VvUBCs slightly decreased or increased during grape ripening.

To compare the expression patterns in leaves and during fruit ripening, all 43 VvUBCs were performed qRT-PCR using Cabernet Sauvignon. Most VvUBCs (22 of 43) showed down-regulated in three stage (EL-33, EL-35, EL-37), especially in VvUBC8 and VvUBC11, which were down-regulated more than 5 fold at EL-37 stage compared to EL-33 stage (Fig. 6). Nine VvUBCs were up-regulated, and three VvUBCs (VvUBC23/24) deceased at veraison stage (EL-35) then increased at EL-37 stage. The transcript level of VvUBC3 peaked at veraison stage then declined until EL-37 stage. Eighteen of 43 VvUBCs showed higher expression level in young leaves than that in berries, especially VvUBC30 and VvUBC45, which approximately were 50 and 400 fold in leaves compared to berries, respectively. Eleven VvUBCs showed lower expression level in young leaves than that in berries (Fig. 6).
Expression analysis of response of VvUBCs to different abiotic stresses. Expression pattern of grape UBC genes under cold and heat stress were investigate using published data SRP018199 and GSE41423 respectively (Fig. S7). Seven (VaUBC3/12/17/25d/31/33) and six members (VvUBC9/10/20/52) responded to cold and heat treatments, respectively. In leaves of Vitis amurensis Rupr., four VaUBCs (VaUBC3/25d/31/33) were significantly down-regulated, and the other three VaUBCs were significantly up-regulated after 4 hours under 4°C cold treatment. Heat treatment was performed under 45°C using Cabernet Sauvignon, and then recovery at control condition. Four VvUBCs (VvUBC9/10/20/52) were detected obviously up-regulated compared with control after heat treatment in leaves, whereas VvUBC11/27 was down-regulated slightly. When recovery after heat treatment, VvUBC9/20/52 showed higher expression in control than treatment groups, whereas VvUBC10/11/27 showed lower. Thirty-three VvUBCs were identified in Cabernet Sauvignon from GSE31677, but these genes show no remarkable change during 16 days of salt or water-deficit stress (Fig. S8).

To confirm the transcriptome results, these UBCs of grape were performed qRT-PCR (Fig. 7 and Fig. 8) in Vitis amurensis and Vitis davidii, respectively. Four VaUBCs (VaUBC15/17/25d/33) continuously decreased during cold treatment (4°C,0–24h), VaUBC3 and VaUBC31 decreased at 8h and then increased at 24h, but VaUBC12 showed no significant change at 8h, but decreased at 24h (Fig. 7). Six VdUBCs (VdUBC9/10/11/20/27/52) were up-regulated in detached leaves after heat treatment (38°C 2h, 47°C 40min, Fig. 8).

Discussion
In this study, 43 VvUBCs were identified in grape, and this number was higher than the 39 discovered in rice, 41 in Arabidopsis, less than 52 in tomato, 50 in human, and 75 in maize. These VvUBCs were divided into five groups based on a phylogenetic tree. VvUBC proteins contained almost similar motifs in one group, especially in...
Figure 4. WGD related gene duplication analyses of VvUBCs. VvUBCs are indicated by vertical orange lines. Red bars denote syntenic regions. Chromosomes were indicated in different colors.

Groups II and V. CDS length accounted for 10.45% of the whole gene length, and their promoter location showed a large difference. Chromosome location showed an uneven distribution of VvUBCs genes in 19 chromosomes, but no VvUBC gene was found in chromosome 10. Whole genome duplication events played a significant role in evolution of many organisms, complex WGD events existed in VvUBCs, indicating that VvUBCs perform various functions during grape development. All these analyses showed large differences between VvUBCs in protein structure, gene structure, and promoter location. VvUBCs in one group may exhibit relative functions.

According to domain analysis, conserved domain UBC exists in all VvUBCs. Protein structure of E2 genes is uncommon in plants, but it is widely used in human research. In humans, E2s can be classified based on existence of additional extensions aside from the UBC domain. These extensions result in functional diversity of E2 genes; this functional diversity is related to subcellular localization and interaction between E2 and E3. In this study, fifteen VvUBCs contained extensions with different roles. N-terminal of VvUBC12 contained an X8 domain and a transmembrane domain. This situation indicates that VvUBC12 may contribute to binding of carbohydrates. Similar to UBE2K in humans, VvUBC27 contained a UBA domain in C-terminal; this UBA domain might be related to ubiquitin binding. However, direct role of UBA remains unclear. Conserved motif analysis showed that all VvUBCs contained at least two UBC/RWD domain motifs whereas consist of different motifs, indicating the VvUBCs identified in this study had conserved features of the E2 genes family, and they might play different function in ubiquitination process.

To gain deeper understanding of putative function of VvUBC, temporal and spatial expression profiles were analyzed. In tomato, E2 genes play an important role in regulation of fruit ripening, as determined by virus-induced gene silencing assay. In grape, most VvUBCs in five Italian varieties change during ripening (Fig. 5), similar expression patterns of VvUBCs were obtained from qRT-PCR in Cabernet Sauvignon (Fig. 6), which indicating that E2 gene family might play extensive roles in grape ripening. VvUBC45 showed different expression profiles in Sangiovese (Fig. 5), indicating its distinct roles in this fruit. Additionally, in Corvina,
VvUBC3/7/12/34 were rapidly up-regulated or down-regulated during grape berry development (Fig. S6), indicating involvement of these genes in fruit ripening. Interestingly, VvUBC3 was up-regulated significantly in post-harvest withering stage and VvUBC7/29/34 down-regulated (Fig. S6), they may play significant roles in post-harvest physiology. Aside from berries, E2 genes also played various roles in other organs. In Arabidopsis, AtUBC22 participates in female gametophyte development41. AtUBC1 and AtUBC2 are ubiquitously expressed in roots, leaves, flowers, and seedlings and activation of FLOWERING LOCUS C allow these genes to repress flowering42. In Corvina, VvUBC11 and VvUBC45 exhibited high expression levels in senescing leaves and winter buds, respectively (Fig. S6), VvUBC30 and VvUBC45 showed high expression in young leaves of Cabernet Sauvignon (Fig. 6). These genes may play different roles in grape development compared with other VvUBCs.

E2 genes from both Arabidopsis and rice were not reported to be induced under cold stress 43. The present study revealed that ZmUBCs changed significantly under cold conditions 16. In Vitis amurensis, seven VaUBCs (VaUBC3/12/15/17/25d/31/33) responded to cold treatment (Fig. S7), and the results were confirmed by qRT-PCR (Fig. 7), but the change tendency of VaUBCs showed a slight difference, which might because of the different cold treatment time. At present, no E2 genes were reported to involve in heat stress. However, expression levels of six UBC genes were obviously changed under heat condition and recovery condition from heat treatment in not only RNA-seq data (Fig. S7) but also qRT-PCR (Fig. 8) in grape in this study. The results indicated that these grape UBC genes might be involved in heat response mechanism in grapes. E2 genes presented different responses to heat and cold stresses. These results indicated that there might be different regulatory mechanisms of ubiquitination in response to heat and cold stresses.

E2 genes in several species were functional under salt or drought. GmUBC2 showed enhanced drought and salt tolerance in soybean18, whereas AtUBC32 was strongly induced by salt stress in Arabidopsis20. Three genes (OsUBC13/15/45) were also up-regulated under salt and drought stresses in rice19. In peanut plants, the physiological water stress induced by polyethylene glycol, high salinity, abscisic acid, or low temperature, changed the expression levels of AhUBC245. Increased transcript levels of CmUBC were observed during drought and salinity stresses in Cucumis melo44. Based on previous transcriptome resources, VvUBCs showed no significant changes in response to drought and salt stresses in grapes (Fig. S8). This result indicated that E2 genes may play different roles in herbaceous and woody plants.

Conclusion

In this study, 43 VvUBC members were identified and divided into five groups based on their phylogenetic tree. Protein and gene sequences, and duplication events were analyzed to predict functional characteristics of VvUBC genes. Transcriptome data and qRT-PCR results presented significant roles of VvUBCs in grape growth, maturity and post-harvest physiology. Additionally, seven and six VvUBCs showed responses to cold and heat stresses, respectively. These responses may contributed to grape resistance mechanism. These results provide new insights into the E2 genes family in woody plants and a solid foundation for further research on grape breeding.
Materials and Methods
Identification of grape E2 family members. Tomato E2 family members were obtained from a previous research, which was used in BLAST search to obtain candidate genes of E2 family in grapes. All protein sequences were obtained from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov). HMM was constructed using sequence data and was used to search UBC proteins in grapes with a
cut-off E-value of 0.001. Then, results of BLAST and HMM searches were merged. Next, candidate UBC protein sequence was scanned again using the domain analysis tool NCBI-Conserved Domain Database (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Finally, 43 UBC proteins were identified in grapes.

**Phylogenetic analysis.** A phylogenetic tree was generated by MEGA 6.0. Protein sequence was aligned by Clustal W. Then, alignment was imported into the MEGA 6.0 software, and phylogenetic tree was constructed using neighbor-joining statistical method with 1000 bootstrap replication.

**Analysis of conserved domain, conserved motif, gene structure and promoters.** According to obtained VvUBC protein sequence, domain analysis of proteins was performed by SMART (http://smart.embl-heidelberg.de/). Then, conserved motifs were analyzed by MEME program (http://meme-suite.org/tools/meme). Furthermore, the motifs obtained were annotated using InterProScan (http://www.ebi.ac.uk/Tools/pfa/iprscan/). Promoter 3.0 was used to annotate grape genome to select the most suitable promoter of VvUBCs. Illustrations of promoter and genes were constructed by Gene Structure Display Server (GSDS) software (http://gsds.cbi.pku.edu.cn/). Introns and exons of VvUBCs were detected in grape genomic annotation, and the diagram was constructed by GSDS. Localization of VvUBCs in chromosome was determined according to grape genomic annotation, and diagram was generated by Mapchart 2.3.

**Gene duplication.** Protein sequence in grape was used for self-BLAST search. Then, BLAST results and documented annotation were combined to analyze duplication of VvUBCs by MCscanX. Finally, a map was drawn by Circos.

**Plant growth and Treatments.** To analyze expression of VvUBC genes in different tissue and fruit ripening, young leaves and berries were sampled from Cabernet Sauvignon (Vitis vinifera), which planted at the Germplasm Repository for Grapevines in the Institute of Botany of the Chinese Academy of Sciences, Beijing, China (39° 54′N, 116° 23′E). The vines were planted in 2007 in south-to-north oriented rows, trained to a fan-shape trellis with single trunk, and subjected to similar management practices for irrigation, fertilization, soil management, pruning, and disease control. Berries were sampled at three developmental stages (EL-33, EL-35, EL-37) according to EL system, each sample was collected from nine clusters, and approximately 20 berries from three clusters formed one biological replicate. Sixth leaves were sampled with three biological replicates.

*Vitis amurensis* were used for cold treatment. Tissue cultured *Vitis amurensis* were grown on half-strength Murashige and Skoog (1/2 MS, pH 5.8) solid medium 1% sucrose and 0.7% agar in conical flasks (120 mL) in a growth chamber at 26°C under a 16-h light/8-h dark photoperiod and 100μmol m⁻² s⁻¹ light intensity.

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**Figure 7.** qRT-PCR results of seven VaUBCs under cold treatment. Data was normalized to VvActin gene expression level. Each VaUBCs at 0 h was normalized as “1”. The mean expression value was calculated from three independent replicates. Vertical bars indicate the standard error of mean. **P < 0.01 and *P < 0.05 compared with 0 h.**
Six-week-old plantlets were subjected to cold stress, plantlets were transferred to a low-temperature chamber at 4 °C with a 16-h light/8-h darkness cycle. The shoot apex with the first fully expanded leaf was harvested at specific time (0 h, 8 h, and 24 h) after initiating the treatments with three biological replicates.

Spine grape (Vitis davidii) was used to analyze expression of VvUBC genes under heat stress. The vines were planted in the same condition as Cabernet Sauvignon introduced above. Detached leaves of approximately 30 days in age were used for heat treatment according to previous study 46. In June of 2017, samples were taken in the morning, placed in the dark with the petiole in water, and then treated by heat stress. The heat stress process was as follows: leaf discs (5.5 cm in diameter) were cut from the detached sample leaves, wrapped in a wet paper towel and placed in a small vessel made of aluminum foil. The vessels were then floated on water in a temperature-controlled water bath, 38 °C 2 h and then 47 °C 40 minutes. Leaves samples were collected with three biological replicates at this time. The control was the same condition as heat treatment except temperature-controlled water bath in 25 °C, and the leaves samples were collected at the same time with three biological replicates.

**RNA extraction and quantitative real-time PCR (qRT-PCR) analysis.** All samples were immediately obtained frozen in liquid nitrogen and stored at −80 °C for RNA extraction. Total RNA was extracted from collected samples using RNeasy Plant Kit (TIANGEN, Beijing, China) following the manufacturer’s procedure. A maximum of 1 μg total RNA was used for synthesizing cDNA by HiScript Q RT SuperMix (Vazyme, Nanjing, China), and the product was subjected to qRT-PCR with an Opticon thermocycler (CFX Connect Real-Time System; Bio-Rad, Hercules, CA) using SYBR Green PCR master mix (Vazyme, Nanjing, China) according to the manufacturer’s instructions. The PCR cycling conditions were as follows: 95 °C for 10 min, 40 cycles of 95 °C for 10 s, 60 °C for 30 s; a 65–95 °C melt curve was analyzed to detect possible primer dimers or nonspecific amplification. VvActin (Accession number: EC969944) was used as stable reference genes. Gene specific primer pairs for qRT-PCR (listed in Table S1) were designed by NCBI Primer BLAST. The specificity of the primers was further verified through gel electrophoresis and reaction product sequencing. Three biological replicates were performed to ensure the accuracy of results. The relative expression of the target genes was determined.
using the 2−ΔΔCt method. All experiments were performed with three biological replicates and three technical replicates. Statistical difference were performed by t-test (*P < 0.01, **P < 0.05, n = 3) using R software.

Transcriptomic resources. Transcriptomic data used in this study were obtained from previous research. Expression levels in different organs were analyzed using GSE36128. A total of 54 organs were collected from grapevines Corvina (Vitis vinifera) for RNA extraction. The entire list of 54 organs can be found as Supplementary Table S2. Three biological replicates were obtained for each sample. Data of four stage of berries in five varieties analysis were obtained from GSE62744. Grape berries were collected from five red-skin grapevine (Vitis vinifera) cultivars (Sangiovese, Barbera, Negro amaro, Refosco, and Primitivo) at four phenological stages (pea size, berry tough, soft, and harvest), with three biological replicates acquired for each sample.

Cold treatment in SRP018199 was performed as follows: Vitis amurensis seedlings were grown in 16 h light/8 h dark photoperiod at 26 °C. These seedlings were then transferred into a chamber at 24 °C under 16 h light at 6:00 am. Cold treatment was started at 9:00 am with constant light. During the first four hours, temperature dropped to 5 °C per hour and was held at 4 °C for an additional four hours. Seedlings used for control were also transferred to growth chambers but without cold treatment. Shoot apices with one well-developed leaf were harvested from three independent replicates. RNAs were isolated for digital expression library construction.

Heat treatment in GSE4423 was conducted as follows: Cabernet Sauvignon (Vitis vinifera) was grown in 25/18 °C day/night condition before treatment. Then, the experimental group was treated at 45 °C from 9:00 to 14:30. Next, leaf samples were obtained and recovered rapidly at 25 °C for 15 min. Leaf samples were collected from the following morning at 9:00. Control group was grown in 25/18 °C day/night condition. Leaf samples were collected from the experimental group.

Cabernet Sauvignon (Vitis vinifera) were treated under water-deficit and salinity stress conditions (GSE31677). This process is listed in Supplemental Table S3.

Data Availability. The datasets analysed during the current study are available from the corresponding author on reasonable request.

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Author Contributions
Z.L. and S.L. designed the research. Y.G. and Y.W. performed the experiments. Y.G., Y.W., and H.X. analyzed data. Y.G., Z.L., S.L. and H.X. wrote the manuscript. All authors read and approved the manuscript.

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