Cloning and Expression Analysis of Seven VvGA2ox Genes in Ovule Development of Seeded and Seedless Grapes (Vitis vinifera)

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Research Article

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Abstract

The GA2ox proteins in plants play important roles in the regulation of growth and development, involving in the conversion of physiologically active gibberellins to inactive gibberellins. In this study, we identified 13 *VvGA2ox* genes in a grape genome-wide analysis and performed bioinformatics analysis for these genes. Phylogenetic analysis of VvGA2ox with homologous in *Arabidopsis*, maize, and peach showed that these protein can be divided into three subfamilies. Gene structure analysis and synteny analysis of *GA2ox* gene family revealed that *VvGA2ox* genes have undergone a complex evolutionary process. Gene expression analysis of tissue-specific for seven *VvGA2ox* genes suggested that these were lower expression in the vigorous growth tissues. Considering that gibberellin can regulate seed development, in order to further determine the influence of *VvGA2ox* on seed development, seven *VvGA2ox* genes were performed with qRT-PCR. The expression profiling of *VvGA2ox* genes in 'Pinot noir' and 'Thompson seedless' during various stages of seed development revealed that *VvGA2ox3c*, *VvGA2ox4* and *VvGA2ox7* may participate in seed-specific regulation. In summary, these results show that *VvGA2ox* has a significant difference between seedless and seeded varieties, which may be related to seedless traits.

1. Introduction

Gibberellins (GAs) as a common plant hormone, widely participate in major aspects of plant growth and development, such as regulating germination and development of seeds, elongation in stem, flowering and fruit-set [1–4]. The GAs in plants were divided into two categories: the bioactive GAs (bio-GAs) and the inactive GAs (in-GAs), only bio-GAs can make efforts. Simultaneously, some catabolic enzymes have been reported to transform the bio-GAs and its precursors into in-GAs, including GA METHYL TRANSFERASE (GAMT) [5], GIBBERELLIN 2 OXIDASE (GA2ox) [6], and CYP714A enzymes [7]. Biosynthetic and catabolic enzymes of GAs control the content of gibberellin in the plant and regulate the plant growth and development. In present, 136 GAs were identified from plants, fungi, and bacteria, only gibberellin 1 (GA_1), gibberellin 3 (GA_3), gibberellin 4 (GA_4) and gibberellin 7 (GA_7) were identified as bioactive forms in plants [4, 8–10]. GA_1, GA_3 and GA_4 are widely present in most plants, while GA_7 is mainly present in fungi [9].

The GA2oxs as a group of key gibberellin oxidase regulate the content of bio-GAs in plants by inactivating the bio-GAs and adjusting the growth and development of plants. The GA2oxs in plants can be divided into three classes, C19-GA2oxs-Class I, C19-GA2oxs-Class II and C20-GA2oxs-Class III. C19-GA2oxs can oxidize C_{19} GAs, C20-GA2oxs accept C_{20} GAs as substrates [11], except this classification rule, some GA2oxs show multifunction, Class III AtGA2ox9 can reduce 2α-hydroxylation of C_{19} GAs and desaturate activity of C_{20} GAs. [12]. In present, GA2oxs have been identified and functionally characterized in various plant species, including *Arabidopsis*, tomato and rice[6, 13, 14]. The changes of GA2oxs expression can regulate the level of endogenous GAs in some plant species. For example, overexpression of *GA2ox* genes in plants causes deficiency in endogenous GAs and leads to plants dwarf [13–15]. The gene silencing of *GA2ox* induced parthenocarpic development and inhibited the lateral branch in tomato [16]. Some GA2oxs could also contribute to cold stress tolerance and fertility in *Arabidopsis*, such as *AtGA2ox9* and *AtGA2ox10* [12]. In grape, VvGA2oxs were differently expressed during inflorescence development without GA application [17], but the relationship between GA2oxs and seedless traits in grape has not been studied.

Exogenous gibberellin treatment contributed to obtain seedless grape. Many studies have shown that exogenous gibberellin can adjust the flowering and fruit set [18], enhance the ability to resist abiotic stress [19] and induce seedlessness [20]. Moreover, the key genes of active gibberellin synthesis and metabolism have been studied, such as *VvGID1A* [21], GA_3 receptor gene [22], *GA20ox*, *GA2ox* [23] and signal components to regulate gibberellin [21].

VvGA2oxs, as the key gene in the metabolic process of active gibberellin, may regulate the development of grape seed development. In this study, we identified and analyzed gibberellin oxidase genes *GA2ox* family in grape, cloned seven of them from 'Pinot noir' and analyze their tissue specific expression in eight different tissues. Moreover, we analyzed the *VvGA2oxs* expression of ovule in seeded and seedless varieties using qRT-PCR. The results provide valuable information on the roles of *VvGA2ox* gene in the seed development and seed abortion of grapevine, and explore the seedlessness mechanism of grape.

2. Materials And Methods

2.1 Plant Materials

'*V. vinifera* cultivars Thompson seedless' and '*V. vinifera* cultivars Pinot noir' grapevine plants were grown in the Grape Germplasm Repository of Northwest Agriculture and Forestry University Yangling, Shaanxi, China. Grape ovules were sampled after the full-bloom stages of 10 d, 15 d, 20 d, 25 d, 30 d, 35 d, 40 d, and 45 d, the same size clusters of *V. vinifera* cv. Thompson seedless and *V. vinifera* cv. 'Pinot noir' were sampled, and the stripped and stored using the methods described previously [24]. The root, stem, leaf, flower, tendril and alabastrum tissues, as well as pericarp and ovule at 30d after full-bloom, were also sampled from '*V. vinifera* cv. Pinot noir' variety and treated as described above.

2.2 Identification of Grape Gibberellin Oxidase Genes *GA2oxs*

The *GA2ox* genes were searched in the *Arabidopsis* database (https://www.arabidopsis.org/), from which the amino acid sequences of *GA2ox* genes were downloaded. The downloaded amino acid sequences were used to search in the Pfam database (https://pfam.xfam.org/search#tabview=tab0), thus acquiring two HMM file of the GA2ox domain, 2OG-FeII_Oxy (PF03171) [25] and DIOX_N (PF14226) [26]. The *GA2ox* gene family members in grape were obtained by blast analysis in the 12× *Vitis* genome (http://plants.ensembl.org/Vitis_vinifera/Tools/Blast), with *E* value less than 0.01. All members of the *GA2ox* gene family in grape were filtered in the SMART databases (http://smart.embl.de/) and Pfam with domain types. The protein annotation information on the *GA2ox* gene family members was studied in Uniport (https://www.uniprot.org/) by searching Uniport ID.

The sequences of CDS, cDNA and amino acids were downloaded from Ensembl Plants (http://plants.ensembl.org/index.html). The online tool ExPASy (https://web.expasy.org/protparam/) was used to predicted the molecular weights (Mw) and isoelectric points (pI) of the *GA2ox* genes in grapevine. SignalP-5.0 Server website (https://services.healthtech.dtu.dk/service.php?SignalP-5.0) was used to analysis signal peptide of the *GA2ox* genes. The intracellular

localizations of GA2ox genes in grape predicted in the softberry website (http://linux1.softberry.com/berry.phtml?topic=protcomppl&group=programs&subgroup=proloc) .

2.3 Phylogenetic Analysis of *GA2ox* Genes

The *GA2ox* genes in *Arabidopsis* were gotten from *Arabidopsis* database by searching GA2ox; the *GA2ox* genes in peach [27] and maize [28] were obtained from the published articles. The amino acid sequences of the *VvGA2ox*, *AtGA2ox*, *PhGA2ox* and *ZmGA2ox* proteins were downloaded from each genome from EnsemblPlants. The GA2ox proteins of grapevine, Arabidopsis, peach and maize were analyzed for multiple sequence alignments using Clustal-omega [29], A Neighbor-joining phylogenetic tree was constructed by the MEGA X software with 500 bootstrap replications [30].

2.4 Structural Analysis of *GA2ox* genes in grape

The phylogenetic tree of the *GA2ox* gene family in grape was generated using the Neighbor-joining method from MEGA X software, with the Bootstrap value set at 1000. *GA2ox* genes motif were analyzed in an online MEME database (https://meme-suite.org/meme/meme_5.3.2/tools/meme) [31]. Intron and exon organizations of grapevine *GA2ox*s were predicted and analyzed using TBtools according *Vitis_vinifera*.12X.51.gff3 file downloaded from EnsemblPlants (http://plants.ensembl.org/Vitis_vinifera/Info/Index) [32]. All results were putted in one picture using TBtools software [32].

2.5 Chromosome Localization and Synteny Analysis of *VvGA2ox* Genes

The locations of the *GA2ox* genes in each chromosome were obtained from 12× *Vitis* genome form EnsemblPlants and the physical localization map of each chromosome was generated using TBtools software.

Genes synteny and collinearity between *V. vinifera* and Arabidopsis and within the grapevine genome were analysis by using MCScanX [33]. The chart was plotted pictures using TBtools software.

2.6 Analysis of the Predicted *cis*-Acting Elements of Promoters of the *VvGA2ox* Genes

The promoter sequences of *GA2ox* in grape were downloaded from the 12× *Vitis* genome database, the length of all *VvGA2oxs* promoter sequences was 2000 bp on the 5' Flanking region (upstream) from initiation codon ATG of *VvGA2ox* genes, the downloaded promoter sequences were submitted to the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/). Results of these predicted cis-acting elements were analyzed and presented as a chart.

2.7 TissueSpecific Expression Analysis of *VvGA2ox* genes

Expression data were retrieved from the GEO databases (https://www.ncbi.nlm.nih.gov/gds/). 54 samples of *V. vinifera* cv. 'Corvina' were studied in dataset GSE36128 [34]. Target genes were screened by R language. Raw datasets were standardized using base-2 logarithms, and the heatmap was constructed using TBtools software.

2.8 RNA Extraction and Primer Design

Total RNA was isolated using the E.Z.N.A®Plant RNA Kit, according to the manufacturer's instructions (OMEGA,China). 2 μg of total RNA from each sample was used for the reverse transcription reaction using FastQuant RT Kit (TIANGEN).

Based on grape genomic sequence and combined GA2ox EST sequences in grape EST database, primers for genes clone and qRT-PCR analysis were designed by Primer premier 5.0. The detailed primers are showed in Table S1.

2.9 Clone of Seven *VvGA2ox* Genes

Gene clone reactions were performed in total volume of 25.0 μL and the conditions for RT-PCR were as follows: 1.0 μL of cDNA, 1.0 μL each of the forward and reverse primers, 2.5 μL of the LATaq buffer (10×), 2 μL of the dNTP mix, 0.5 μL of LA Taq DNA polymerase, and 17 μL of ddH_2O. The thermal parameters of PCR were as follows: 94 ˚C for 3 min, 94 ˚C for 30 s, 55 ˚C for 30 s, and 72 ˚C for 2 min for 37cycles, and 72 ˚C for 10 min. The PCR products were ligated with the pMD19-T vector (Takara) and then transformed into *Escherichia coli* TOP10. Finally, the products were sequenced after PCR screening of bacterial colonies, each gene was performed in 3 replicates.

2.10 Quantitative Real-Time-PCR expression analysis of Seven *VvGA2ox* Genes

Total RNA and first strand cDNAs were prepared as described above. The Primer5 software was used to design the specific primers used for qRT-PCR, and actin as an internal control gene. qRT-PCR was conducted using iQ5 Real-Time PCR Detection System. The conditions for qRT-PCR were as follows: 1.0μL of cDNA, 0.8 μL each of the forward and reverse primers, 10.0 μL of SYBR Green, 7.4 μL of ddH_2O. The thermal parameters for qRT-PCR were: 95 ˚C for 1 min, 45 cycles of 95 ˚C for 20 s, 59 ˚C for 20 s, and 72 ˚C for 30 s.

3. Results

3.1 Identification and Phylogenetic Analysis of *GA2ox* Genes in Grapevine

A total of thirteen candidate GA2ox genes were identified in grape (Table 1). In order to confirm the *GA2oxs* family members, the domains of candidate *GA2ox* genes were examined through the Pfam and SMART databases. Family members were named according to phylogenetic analysis between *Arabidopsis* and grape (Fig. S2) [35], annotation information of the *GA2ox* genes was summarized using online tools (Table 1). The cDNA of *GA2ox* genes was from 672bp (*VvGA2ox8b*) to 1753bp (*VvGA2ox7*), but the CDS of thirteen *GA2ox* genes was with large differences that between 672bp (*VvGA2ox8b*) to 1104bp

(*VvGA2ox8c*). The isoelectric point (pI) of them was 5.42 (*VvGA2ox4*)-8.22 (*VvGA2ox3c*). The most *GA2ox* genes have no signal peptide, except *VvGA2ox8c*. The prediction of subcellular localization shows that the *GA2ox* genes are mainly distributed in the extracellular but *VvGA2ox11* distributes in cytoplasmic, which implies *VvGA2ox11* may work specially with other *GA2oxs*.

Table 1
Gene and sequences initial analysis of *GA2ox* in *Vitis vinifera*

Gene Name	cDNA/bp	CDS/bp	Protein Length/aa	pI	MW/kD	Chromosomes locus	Position	Gene ID	UniProt ID	Signal peptide	Sub loca
VvGA2ox3a	994	828	275	5.42	37.27	10	5846741–5848388	VIT_10s0003g03490	D7TK65	-	Extr
VvGA2ox3b	1223	972	323	6.54	36.12	19	15497729–15499650	VIT_19s0140g00120	D7TRA0	-	Extr
VvGA2ox3c	1138	999	332	8.22	37.17	19	15603207–15604930	VIT_19s0140g00140	D7TRA1	-	Extr
VvGA2ox4	1187	1020	399	5.40	37.34	5	343990–346779	VIT_05s0077g00520	D7SYH4	-	Extr
VvGA2ox6	1008	1002	333	6.34	37.07	7	4377203–4380857	VIT_07s0005g01920	A5BYJ8	-	Extr
VvGA2ox7	1753	1023	340	5.65	41.12	1	17965019–17966964	VIT_01s0010g01650	F6HG07	-	Extr
VvGA2ox8a	1628	1035	344	5.62	39.3	10	190065–195087	VIT_10s0116g00410	F6H7K2	-	Extr
VvGA2ox8b	672	672	223	7.09	25.47	19	5851478–5864139	VIT_19S0177G0002	D7T0A2	-	Extr
VvGA2ox8c	1287	1104	367	6.15	42.22	19	5864698–5871371	VIT_19s0177g00030	F6H9A1	+	Extr
VvGA2ox10	1197	1014	337	5.55	37.89	6	7476195–7479644	VIT_06s0004g06790	D7SJW0	-	Extr
VvGA2ox11	1282	1059	352	6.11	39.12	3	15307070–15312521	VIT_03s0017g00620	D7TU51	-	Cyto
VvGA2ox12	1292	1029	342	5.67	39.22	3	4642801–4644279	VIT_03s0063g01150	D7TPQ0	-	Extr
VvGA2ox13	1229	1029	342	5.92	39.19	3	4723791–4725151	VIT_03s0063g01260	D7TPR0	-	Extr

Notes: complementary DNA (cDNA), sequence coding for aminoacids in protein (CDS), isoelectric point (pI), molecular weight (Mw).

3.2 Multiple-Sequence Alignments of *GA2ox* Genes

Sequence similarity matrix analysis of grapevine *GA2oxs* amino acid sequence revealed that the sequence identity between *VvGA2ox*s ranged from 13.21–94.15% (Table S2). The identity between *VvGA2ox12* and *VvGA2ox13* was as high as 94.15%, the lowest identity was 13.21% between *VvGA2ox6* and *VvGA2ox8b*. *VvGA2ox11*, *VvGA2ox12* and *VvGA2ox13* showed the greatest difference with other gene family numbers.

Compared the amino acid sequences among grapevine *GA2ox*s and the 9 known *Arabidopsis GA2ox*s (*AtGA2ox*1-*AtGA2ox4* and *AtGA2ox6*-*AtGA2ox10*), the conserved structures were analyzed in NCBI Conserved Domain Search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). The result showed that the 22 GA2ox protein sequences had a similar conserved structure (Fig. S1). All of them contained a DIOX_N structure domain and a 2OG-Fe□_Oxy structural domain, except AtGA2ox8. Some of GA2ox lost some amino acids in the C-terminal of 2OG-Fe□_Oxy structural domain, they respectively were *AtGA2ox9*, *AtGA2ox2* and *VvGA2ox8a*. The conserved structure analysis showed the two typical GA2ox structural units might play an important role in determining their structures and enzyme activities.

3.3 Gene Structure and Phylogenetic Analysis of Grapevine *GA2ox* Genes

A phylogenetic tree was built using GA2ox protein sequences in grape (Fig. 1a), conservative motif analysis was performed using MEME to obtain 14 conserved motifs (Fig. 1a) and named motif 1 to motif 14 (Fig. S3). Intron and exon organizations of the grapevine *GA2oxs* were predicted according to gene annotation of *Vitis_vinifera*.12X.51.gff3 file (Fig. 1a). The same motifs were found in *VvGA2ox3a*, *VvGA2ox3b*, *VvGA2ox3c*, *VvGA2ox4*, and *VvGA2ox6*. Motifs 1, 2, 3, 4, 6 and 7 were conserved sequences shared by all of the genes. Many of *VvGA2ox* genes contained 3 exons and 2 introns, namely *VvGA2ox3a*, *VvGA2ox3b*, *VvGA2ox3c*, *VvGA2ox4*, *VvGA2ox6*, *VvGA2ox8b*, *VvGA2ox10*, *VvGA2ox12* and *VvGA2ox13*. The remaining gene *VvGA2ox8c*, *VvGA2ox8a*, and *VvGA2ox11* displayed 5 exon/4 intron, 3 exon/3 intron, and 4 exons/3 introns structures, respectively. The conservatism of C19-GA2ox-Class I genes was better than C19-GA2ox-Class II and C20-GA2ox-Class I in grape. The difference of structures may be related to their respective features and differential evolutionary processes.

The *GA2ox* family was divided into three different groups. In order to predict the classification of *GA2ox* genes in grape, we collected reported *GA2ox* sequences from *Arabidopsis*, peach [27] and maize [28] to construct phylogenetic tree with those identified *VvGA2ox* genes by neighbor-joining method

(Fig. 1b). Gene accession numbers of all sequences used in this tree were listed in Supplementary Table S3. The phylogenetic tree showed that the different evolution of 3 subgroups GA2oxs among those species, GA19-GA2ox-Class I in maize revealed an obvious segregation among GA19-GA2ox-Class I enzymes, the familiar feature showed *VvGA2ox11*, *12*, *13* with other GA19-GA2ox-Class II enzymes, GA20-GA2ox-Class I enzymes among those species showed the closer evolutionary relationship. *VvGA2ox7*, *VvGA2ox8a*, *VvGA2ox8c*, *VvGA2ox8b* and *VvGA2ox10* were classed into GA20-GA2ox-Class I. GA19-GA2ox-Class II contained five *VvGA2ox* genes, they were *VvGA2ox4*, *VvGA2ox6*, *VvGA2ox11, VvGA2ox12* and *VvGA2ox13*, respectively. *VvGA2ox3a*, *VvGA2ox3b* and *VvGA2ox3c* were clustered into GA19-GA2ox-Class I. This result predicted that the most of VvGA2oxs in grape might interact with C_{19} GAs.

3.4 Chromosome Mapping and Synteny Analysis of *VvGA2ox* Genes

The 13 VvGA2oxs were distributed unevenly in 7 chromosomes. *VvGA2ox3b*, *VvGA2ox3c*, *VvGA2ox8b*, and *VvGA2ox8c* were located on the 19th chromosome, *VvGA2ox11*, *VvGA2ox12* and *VvGA2ox13* were distributed in the 3rd chromosome, *VvGA2ox8a* and *VvGA2ox3a* were mapped on the 10th chromosome, the remaining four genes were evenly distributed in the 7th, 1st, 5th, and 6th chromosomes (Fig. 2a). Partial genes were very close to each other, such as *VvGA2ox8b* and *VvGA2ox8c*, *VvGA2ox12* and *VvGA2ox13*, *VvGA2ox3b* and *VvGA2ox3c*.

In order to obtain a further understanding of the origin and evolutionary relationship of the *VvGA2ox* genes, a synteny analysis was performed between *V. vinifera* and *Arabidopsis* (Fig. 2b). Five orthologous gene pairs were identified between *V. vinifera* and *Arabidopsis*. The orthology analysis suggested that these pairs of *VvGA2ox* and *AtGA2ox* genes were descended from a common evolutionary ancestor. In addition, only two paralogous *VvGA2ox* gene pairs were found in grapevine (Fig. 2c), *VvGA2ox8a* and *VvGA2ox8b*, *VvGA2ox4* and *VvGA2ox6*.

3.5 Analysis of the Predicted *cis*Acting Elements of Promoters of *VvGA2ox* Genes

The promoters of *VvGA2ox* genes were analyzed in the Plant Care database (Table S4). Except core promoter element TATA-box and some unnamed elements, there are many *cis*-acting elements related to the light response in *VvGA2ox* gene promoters (Fig. 3), especially, Box-4, G-box and GT1-motif existed in most promoters. Other *cis*-acting elements were related to the stress response, development and hormone response. It was noteworthy that the RY-element and GCN4_motif are involved in seed-specific regulation and endosperm expression existed in *VvGA2ox7* and *VvGA2ox12*, respectively (Fig. 3), indicating that they may play an important role in the process of ovule abortion in seedless grapevines. Moreover, the similarity of promoter elements between different *VvGA2ox* genes was lower, which showed that the functional differentiation of these genes was obvious, and these gene may be involved in different physiological processes and stress responses.

3.6 TissueSpecific Expression Analysis of *VvGA2ox* genes

Tissue-specific expression patterns of *VvGA2oxs* in grapevine were retrieved from a published gene expression dataset (GSE36128), the details of these *VvGA2oxs* were provided in Table S5. A heatmap of *VvGA2oxs* in 54 different tissues were showed in Fig. 4a. We found that most *VvGA2oxs* were relatively low expressed in many tissues. Some genes exhibited relatively high in the specific tissues. *VvGA2ox3b* and *VvGA2ox3c* showed generally higher expression levels in summer bud and rachis. *VvGA2ox3b* also exhibited a relatively higher level in tendril. *VvGA2ox8a* was highly expressed in young leave. *VvGA2ox8b*, *8c* exhibited relatively low expression in all tissues. Furthermore, *VvGA2ox3a* showed a significant expression compared with other genes in seed.

Besides, in order to further analyze *GA2ox*s tissue expression, we cloned seven *VvGA2ox* genes from three different subgroups (Fig. 4b) and analyzed their expression in eight different tissues and organs using qRT-PCR (Fig. 4c) *VvGA2ox3a* expressed a high level in seed and summer bud tissues and consisted with the result showed in gene expression dataset (GSE36128). *VvGA2ox3c* expressed a high level in flower and alabastrum, however, its expression level was very low in roots and pericarp. *VvGA2ox4* only expressed a high level in seed. The expression of *VvGA2ox6* was high in roots, stems and buds. *VvGA2ox7* exhibited a higher level in root and its expression was relatively high in seeds. The expression of *VvGA2ox8c* was higher in tendril and leaf. *VvGA2ox8a* was specially expressed in leaf and its expression level was very low in other tissues. Considering *VvGA2ox* involved in the deactivation process of bioactive gibberellin in grapes, the *VvGA2oxs* expressed a low level in flowers and roots etc, except *VvGA2ox3c*, which was is in contrast to expression of gibberellin 3-oxidase in plants, showed that gibberellin was more easily synthesized in tissues with vigorous growth [36].

Most cloned cDNA sequences of seven selected genes were consistent with predicted sequences, but *VvGA2ox3a* showed different sequences in 3' end, *VvGA2ox7* with different sequences in 5' end. All cloned cDNA sequences had been submitted to GenBank and gotten accession numbers: KX238900 (*VvGA2ox3a*); KX238896 (*VvGA2ox3c*); KX238898 (*VvGA2ox4*); KX238894 (*VvGA2ox6*); KX238897 (*VvGA2ox7*); KX238899 (*VvGA2ox8c*); KX238895 (*VvGA2ox8a*).

3.7 Expression Analysis of Seven Grapevine *GA2ox* Genes During Seed Development

The changing expression levels of the seven selected *VvGA2oxs* from three different subgroups during the development of grapevine were analyzed using qRT-PCR (Fig. 11). The expression trend of *VvGA2ox3a* and *VvGA2ox6* were similar in 'Pinot noir' and 'Thompson seedless', whose relative expression level increased extremely at the after full bloom of 10 to 20 DAY and decreased to a low expression with seed development. However, the expression patterns of *VvGA2ox3c*, *VvGA2ox4*, *VvGA2ox7*, *VvGA2ox8a* and *VvGA2ox8c* were different in seeded vs. seedless grapevines. A rapid increasing expression of *VvGA2ox4* happened in 'Thompson seedless' during 10 to 25 DAY, while the expression level of *VvGA2ox4* in 'Pinot noir' was low. For *VvGA2ox8a* and *VvGA2ox8c*, the relative expression levels were on an upward trend in 'Thompson seedless' with seed development, but reversed in 'Pinot noir'. The expression levels of *VvGA2ox3c* and *VvGA2ox7* in 'Thompson seedless' were always higher than in 'Pinot noir' during the seed development. What's more, in a previous experiment, *VvGA2ox7* was predicted to include the RY-element that is closely related to seedspecific regulation. The gene promoter elements and relative expression analysis show *VvGA2ox7* may be related to the ovule abortion in seedless grapevines.

4. Discussion

GA is a plant growth regulator and play a vital role in the process of plant growth and development [37]. The expression of GAs synthesis genes in the vigorous growth tissues is much higher than other organizations [9]. *GA2ox*s are the key gene in the deactivation of bio-GAs or its precursors, and regulate the content of gibberellin in plants [2].

In this study, we identified thirteen *VvGA2ox* genes and performed bioinformatics analysis on them. Eleven *VvGA2ox* genes had been reported, two new *VvGA2ox* genes had been found [23, 38], *VvGA2ox8b* and *VvGA2ox10* were first identified in this paper. Using online tools, we found most GA2oxs were distributed in the extracellular and did not contain signal peptide. It was great different in similarity matrix analysis of 13 *VvGA2ox* genes amino acid sequences, especial GA19-GA2ox-Class and GA20-GA2ox-Class. The great difference identity implied that the evolution of *GA2ox* gene in grape might be different [23, 38] and decided to choose different kinds of GAs (C_{19}-GAs and C_{20}-GAs) as substrate. The intricate evolution of GA2ox genes also showed in gene structural characteristics and phylogenetic analysis among grape, maize, peach, *Arabidopsis*. Different gene structures of *GA2ox8a*, *GA2ox11* and *GA2ox8c* implied that they experienced the evolutionary divergence [39]. Phylogenetic analysis showed that there were different evolution models among 3 subgroups of GA2oxs. The synteny analysis further revealed the evolutionary diversity of *GA2ox* family in grape. Five orthologous gene pairs were found between *V. vinifera* and *Arabidopsis*, two orthologous gene pairs were exhibited in grapevine. Moreover, according to the structural domain analysis, we could find that all of VvGA2oxs contained a DIOX_N structure domain and a 2OG-Fe□_Oxy structure domain, except VvGA2ox8a, which only contained the N-terminal of 2OG-Fe□_Oxy structure domain. These results indicated that *GA2ox* genes in grape have evolved in many ways, but its function in the deactivation of bioactive gibberellin was conserved, and DIOX_N structure domain and N-terminal of 2OG-Fe□_Oxy structure domain were necessary unit for GA2ox enzymes function [40].

Tissue specific expression analysis showed the differential expression pattern of *GA2oxs* in various tissues. The specific GA 2-oxidase transcripts in different tissues suggested that they may have specific functions. In this study, we choose seven *GA2oxs* to clone and analyze their relative expression levels in eight organs. *VvGA2ox3c* might take part in oxidizing bio-GAs in different tissue with the high relative expression, the highest transcript in the flower was consistent with the previous study, in which the content of bio-GAs declined in inflorescence after anthesis [23]. Active GA-deactivating activities during seeds approaching maturation can be used to explain the high expression of *VvGA2ox3a*, *VvGA2ox4* in ovule [9, 41]. Previous studies revealed that GAs regulated leaf expansion and senescence and the developing leaf with high GAs level [9, 42], *VvGA2ox8a* with high expression in leaves may inhibit the growth of grape leaves and accelerate leaf senescence of grape.

Many researches had been reported that gibberellin participated in the regulation of seed development [43, 44] and interacted with other hormones to regulate the seed development of seeded and seedless grapes [44]. The seed abortion is a unique phenomenon of the stenospermocarpy grape that its seed cannot get completed development. The seed abortion trait can be regulated by GAs content of seed, the higher GA3 level of seed usually showed in seedless grapes rather than seeded grapes [45]. Furthermore, exogenous GA3 treatment can induce seed abortion through decreasing in antioxidant enzymatic activities and alter the expression of genes related to seed development, rather than the fertilization [46]. GA2ox enzymes contribute to GAs catabolism and regulate the bio-GAs level in grapevine [23], the high content bio-GAs can activate the transcription of *GA2oxs* [6, 27]. In this study, four *GA2oxs* transcripts were relatively higher in 'Thompson seedless' compared with 'Pinot noir', in agreement with the GA2ox genes upregulated in seedless grapes [45]. Ovule abortion of 'Thompson seedless' happened during 35d-40 d after full bloom [47]. During this period, the upregulated expression of *VvGA2ox3a, 3c*, *4*, *7, 8a* in 'Thompson seedless' promoted gibberellin metabolism, and decreased the content of gibberellin and inhibited the ovule development. In contrast, *VvGA2ox3a*, *3c*, *4*, *6*, *7*, *8c* were downregulated in 'Pinot noir', and provided sufficient GAs to promote ovule development. Moreover, the expression of all *VvGA2ox* declined to the lowest level at 45 DAF when the ovule abortion of 'Thompson seedless' completed. This trend of expression change indicted that *VvGA2ox*s had an impact on the development of grape ovule.

In present, some *GA2oxs* have shown their special functions in seed development. Ectopic expression of a pea GA 2-oxidase2 cDNA in *Arabidopsis* resulted seed abortion [48]. Overexpression of *PsGA2ox2* with a MEDEA promoter in *Arabidopsis* caused seed aborting and seed numbers reducing [49]. In tomato fruit, specially overexpressed of *SlGA2ox1* reduced the number of seeds and seed germination rate [13]. The transgenic rice of overexpression *OsGA2ox1* failed to set grain [50]. And the loss-of-function mutants of *AtGA2ox10* in *Arabidopsis*, increased number of seeds per silique [12]. In summary, the relationship between bioactive gibberellin and grape ovule abortion is very obvious. GA2ox enzymes accept bio-GAs as substrate, which are the key gene to participate in GA-mediated ovule abortion. The identification and expression analysis of grape *GA2ox* genes will provide a basis for the further study, to explore whether bioactive gibberellin is related to the abortion of grape ovules.

Declarations

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Conflict of interest statement

The authors declare that they have no conflict of interest.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Chaohong Zhang, Jixiang Kong, Ting Zhao and Bingchen Liu. The first draft of the manuscript was written by Jixiang Kong and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

All the authors have read and consented to submit the manuscript.

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Figures

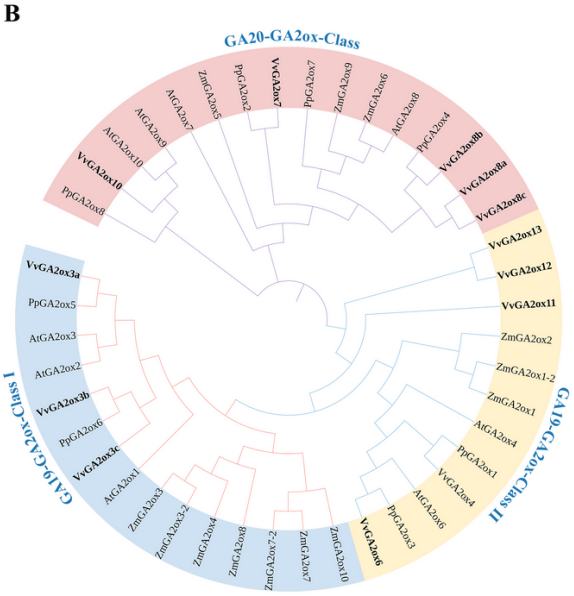

Figure 1

Phylogenetic tree analysis of GA2oxs. **a** Phylogenetic and structure analyses of *VvGA2ox* genes. Left: phylogenetic analysis; Middle: gene intron/exon structures analysis; Right: motif analysis. **b** Phylogenetic tree of the predicted amino acid sequences GA2oxs from different species. The pink range represented the subgroup of GA20-GA2ox-Class, the orange range represented the subgroup of GA19-GA2ox-Class II, the purple range represented the subgroup of GA19-GA2ox-Class II

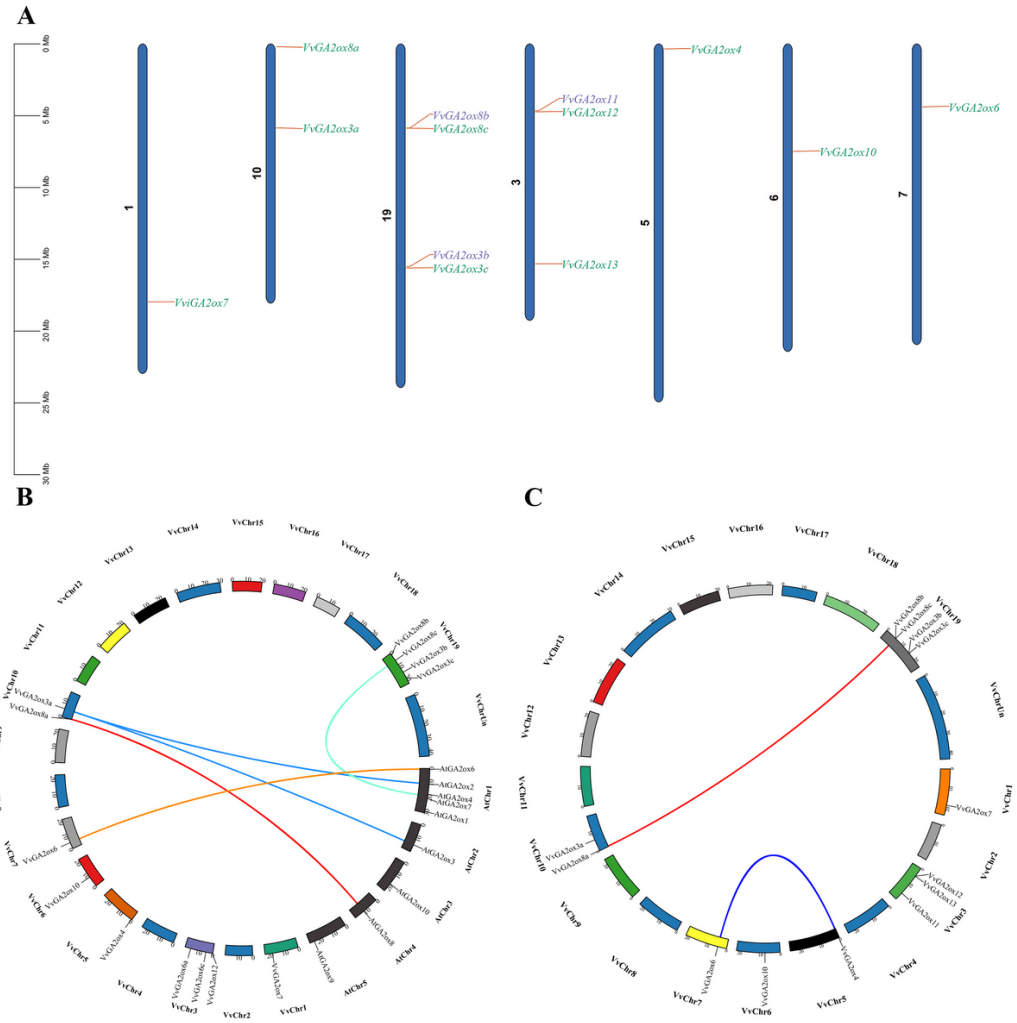

Figure 2

Chromosome Mapping and Synteny Analysis of *VvGA2ox* Genes. **a** Chromosome localization of *VvGA2ox* genes. The vertical blue bars of various lengths represent the grape chromosomes. The oblique black lines indicate the positions of the *VvGA2ox* genes (Color figure online) **b** Synteny analyses of *GA2ox* genes in grapevine and *Arabidopsis*. The black lines on the circle indicate the approximate chromosome location of *GA2ox* genes in grapevine or *Arabidopsis*. Syntenic pair genes between *V. vinifera* and *Arabidopsis GA2ox* genes are represented by lines of various colors **c** Synteny analyses of *GA2ox* genes within grapevine. The black lines on the circle indicate the approximate chromosome location of *GA2ox* genes in grapevine. Syntenic pair genes in *V. vinifera GA2ox* genes are represented by lines of various colors

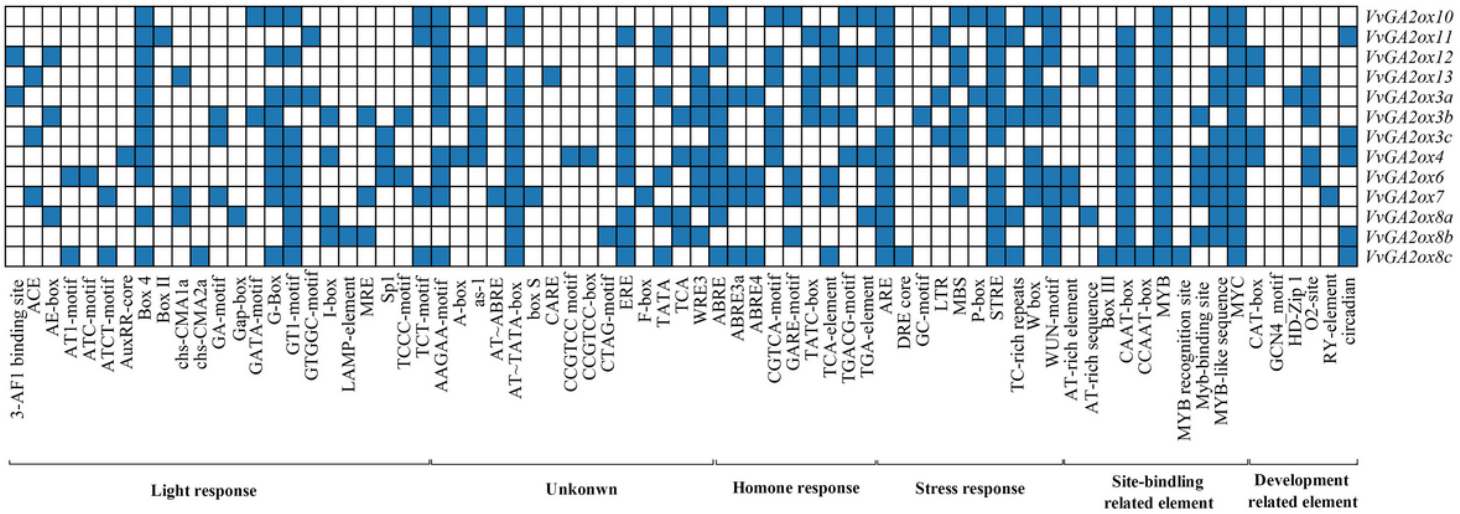

Figure 3

The relevant results of the predicted elements of promoters of *VvGA2ox* genes from the Plant Care database. The horizontal axis shows the kind of element. The blue squares indicate the element is predicted in the *VvGA2ox* gene promoter, while the white squares indicate this kind of element is not included in the gene promoter

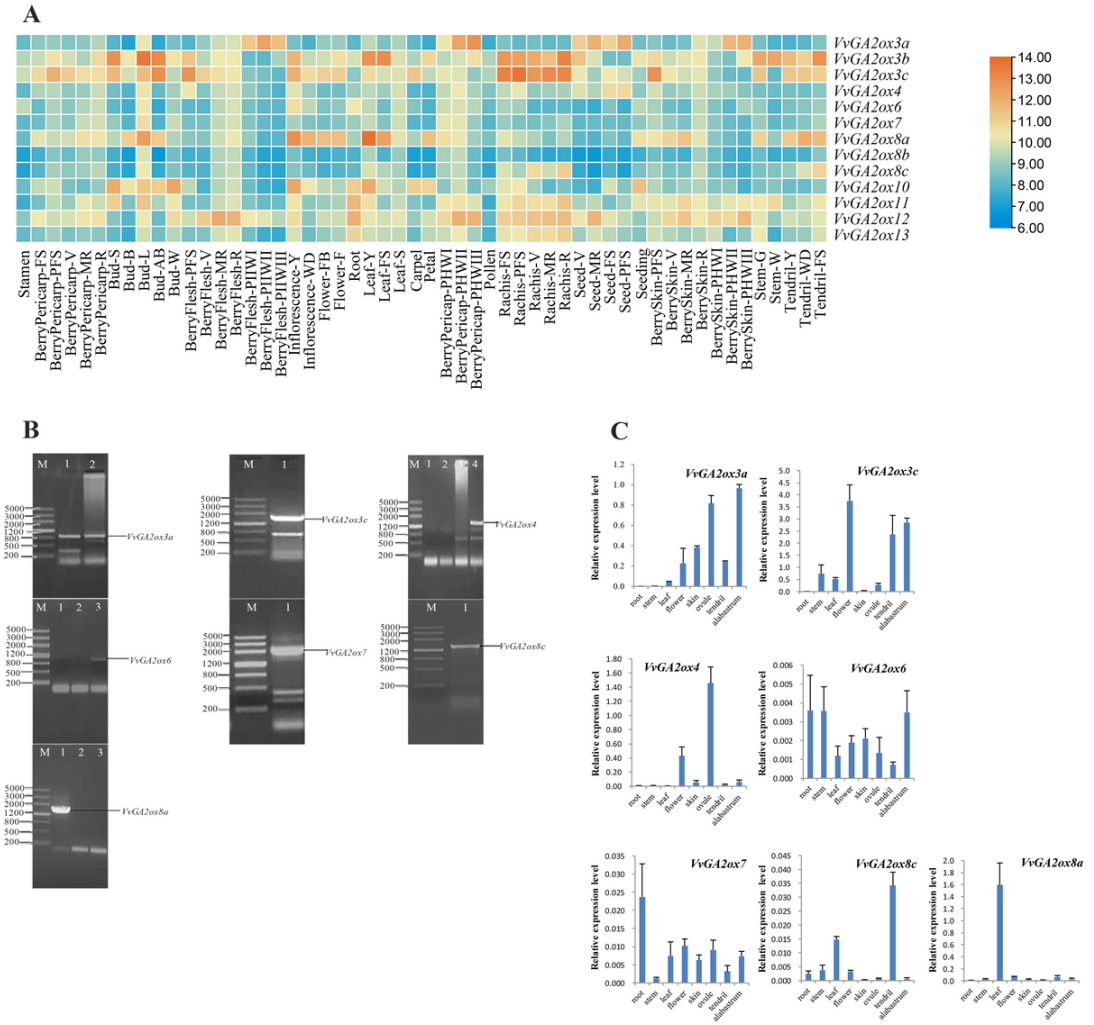

Figure 4

Tissue specific expression analysis of VvGA2ox genes. **a** The tissue-specific expressions of *VvGA2ox* genes based on the GSE36128. Expression data were processed with log2 normalization. The horizontal axis shows 54 tissues and organs. Different colors in the cells correspond to different scores indicating the expression level of the gene in the specific tissue (see key above). The higher the score, the higher the expression level of the gene in the specific tissue **b** The electrophoresis pattern of the cloned seven *GA2ox* genes in *V. vinifera*. M represented DNA Marker, the numbers represented the different swim lane **c** Relative gene expression of seven *GA2ox* genes in eight different tissues or organs of 'Pinot noir' (root, stem, leaf, flower, skin, seed, tendril and alabastrum). Y axes represented relative expression levels, error bars above showed ± SD

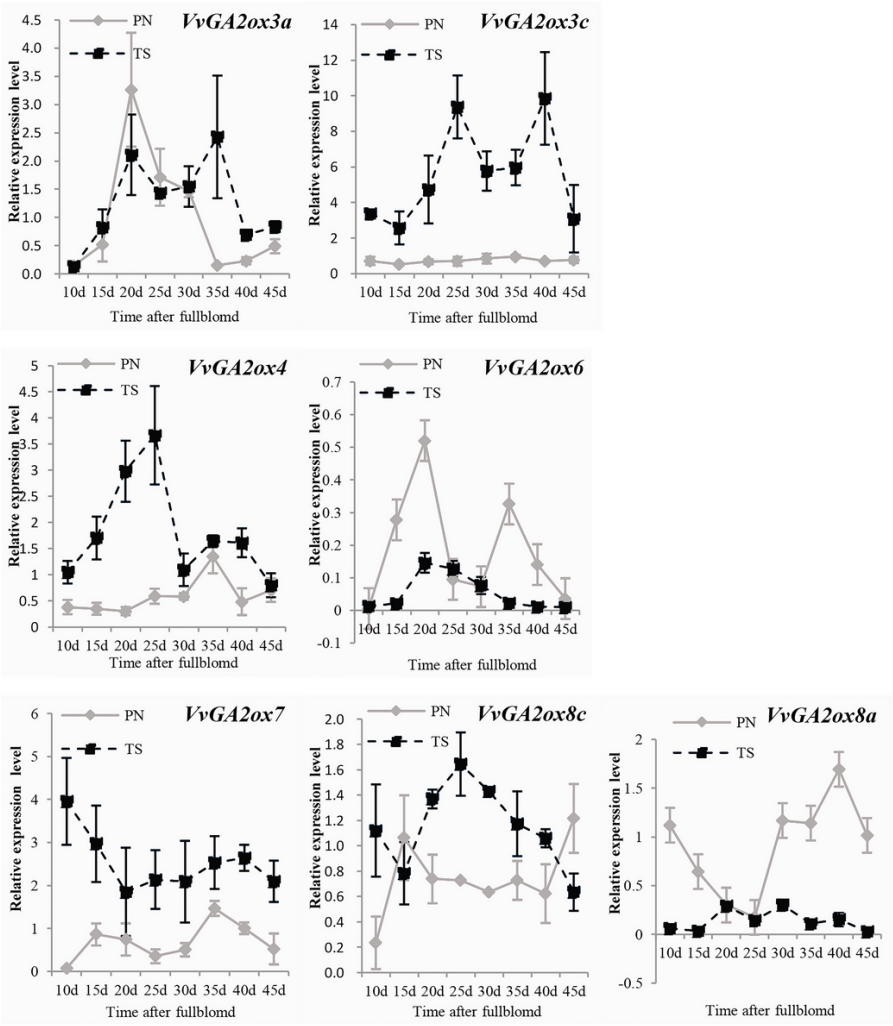

Figure 5

Relative gene expression of GA2ox genes analyzed by qRT-PCR indifferent stages of ovule development in 'Thompson seedless' and 'Pinot noir'. Blue line represented the changes of GA2ox genes' relative gene expression in 'Thompson seedless', red line represented the changes of GA2ox genes' relative gene expression in 'Pinot noir'. Y axes represented relative expression levels, error bars above showed ± SD

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