**Characterization of GATA gene family in *Vitis vinifera*:
genome-wide analysis, expression profiles, and involvement
in light and phytohormone response**

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1 **Characterization of GATA gene family in *Vitis vinifera*: genome-wide**
2 **analysis, expression profiles, and involvement in light and**
3 **phytohormone response**

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Abstract

The plant GATA family is one of the most important transcription factors involved in light-responsive development, nitrogen metabolism, phytohormone signaling, and source/sink balance. However, the function of the *GATA* gene is less known in grape (*Vitis vinifera* L.). In this study, we comprehensively analyzed the GATA family in grape, particularly the phylogenetic evolution, duplication patterns, conserved motifs, gene structures, *cis*-elements, tissue expression patterns, and predicted function of *VvGATA* genes in response to abiotic stress. The potential roles of *VvGATA* genes in berry development were also investigated. The GATA transcription factors displayed expression diversity among different grape organs and tissues, and some of them showed preferential expression in a specific tissue. Heterotrophic cultured cells were used as model systems for the functional characterization of the *VvGATA* gene and study of its response to light and phytohormone. Results indicated that some *VvGATA* genes displayed differential responses to light and phytohormones, suggesting their role in light and hormone signaling pathways. A thorough analysis of GATA transcription factors in grape (*V. vinifera* L.) presented the characterization and functional prediction of *VvGATA* genes. The data presented here lay the foundation for further functional studies of grape GATA transcription factors.

Keywords Grape (*Vitis vinifera* L.), GATA transcription factor, Evolution, Expression analysis

Introduction

The zinc-finger domain is found in many proteins involved in protein–DNA interactions; GATA transcription factors encode a type IV zinc-finger protein that binds to the DNA sequence (A/T)GATA(A/G) and acts as regulators of gene expression (Pavletich and Pabo 1991; Lowry and Atchley 2000). GATA transcription factors are encoded by small multigene families, and their protein sequences contain either one or two conserved CX₂CX_{17–18}CX₂C motif (Teakle and Gilmartin 1998). Animal GATA factors were originally identified to contain CX₂CX₁₇CX₂C domains, and the first GATA gene *Eryfl* was identified in animals and found to activate all erythroid-specific genes (Evans et al. 1988; dEvans and Felsenfeld 1989; Tsai et al. 1989). Subsequent research revealed that GATA factors are critical to the growth and development of vertebrates and invertebrates (Patient and McGhee 2002; Wlodarski et al. 2017; Fujiwara 2017). A fungal GATA factor generally contains a CX₂CX₁₇CX₂C or CX₂CX₁₈CX₂C domain and is involved in nitrogen metabolism or light response (Teakle and Gilmartin 1998; Wong et al. 2008; Hunter et al. 2014; Niehaus et al. 2017). In plants, GATA factors were originally identified in the regulatory regions of light-responsive genes; some light-inducible genes contain GATA motifs in their promoter regions, and an individual GATA motif that combines with other light-responsive elements and responds to light signals (Puente et al. 1996; Argüello-Astorga and Herrera-Estrella 1998; Chattopadhyay et al. 1998; Teakle et

al. 2002; Jeong and Shih 2003). The first plant *GATA* gene (*NTL1*) was cloned from tobacco (*Nicotiana tabacum*). A foundation study predicted the existence of 29 and 28 *GATA* genes in *Arabidopsis* and rice, respectively. Furthermore, most of plant *GATA* factors contain a CX₂CX₁₈CX₂C domain, and several genes contain a CX₂CX₂₀CX₂C motif or encode a two-zinc finger domain, according to structural analysis results (Daniel-Vedele and Caboche 1993; Reyes et al. 2004).

In higher plants, *GATA* genes are involved in various biological processes, including light response, nitrogen metabolism, chloroplast biogenesis, germination, floral development, and phytohormone signaling. The *Arabidopsis GATA2* is a key light-signaling transcription factor that mediates photomorphogenesis (Luo et al. 2010), and *GNC* and *CGA1* are master transcriptional regulators involved in *Arabidopsis* chloroplast biogenesis (Chiang et al. 2012). B-*GATA* genes, one of the *GATA* factor subfamilies from *Arabidopsis*, have been recently characterized in multiple developmental processes. For instance, the LLM-domain B-*GATA* genes *GNC* and *CGA1/GNL* control germination, flowering, and senescence; some LLM-domain B-*GATA* genes play important roles in greening, floral development, senescence, cell divisions, and stomata formation (Behringer et al. 2014; Behringer and Schwechheimer 2015; Ranftl et al. 2016; Klermund et al. 2016). The HAN-(HANABATARANU) domain B-*GATA* gene (*HAN*) is required in early embryo development of *Arabidopsis* (Nawy et al. 2010). In monocot rice, *NLI* plays an important role in organ differentiation and reproductive development (Wang et al. 2009). *OsGATA12* regulates chlorophyll content, senescence, and yield in

85 rice (Lu et al. 2017). In woody plant species, poplar GATA transcription factor *PdGNC*
86 regulates chloroplast ultrastructure and photosynthesis (An et al. 2014). Furthermore,
87 GATA factors are responsive to hormone signals, such as auxin and gibberellin signals,
88 which regulate the downstream target genes *GNC* and *GNL* during plant growth and
89 development; these signals also regulate brassinosteroid, which participates in the
90 regulation of the GATA transcription factor *GATA2* during *Arabidopsis*
91 photomorphogenesis (Luo et al. 2010; Richter et al. 2013).

92 Grape is one of the major fruit crops in the world due to its huge economic value.
93 Reports on the GATA transcription factors in grape are few. To elucidate and explore the
94 functions of the grape GATA transcription factors, we performed a genome-wide analysis
95 and identified 19 grape GATA genes (*VvGATA*) in this study. Then, we analyzed
96 phylogenetic relationships, conserved motifs, gene structures, *cis*-elements, and gene
97 duplication events in grape GATA transcription factors. The expression patterns of
98 *VvGATA* in different grape organs were investigated through quantitative real-time PCR
99 (qPCR) and RNA sequencing (RNA-seq). We also examined their expression profiles in
100 response to light and phytohormones. Its subcellular localization was also identified by
101 protoplast transfection. These studies will provide wider insight on the function of
102 *VvGATA* in grape.

103 **Methods**

104 **Sequence retrieval and identification of *VvGATA* genes**

105 Analyses of the predicted DNA sequences and protein sequences of *V. vinifera* were

performed using the HMMER software (<http://www.hmmerr.org/>). First, we downloaded the *Arabidopsis* GATA protein sequences from the Arabidopsis Information Resource (<http://www.arabidopsis.org/>) according to a previously described sequence database of *Arabidopsis* (Reyes et al. 2004; Manfield et al. 2007). Then, we constructed a Hidden Markov Model to search for GATA sequences and used it to initially obtained 23 *V. vinifera* GATA protein sequences from the grape genome sequence. All putative GATA protein sequences in the conserved domains were manually analyzed and confirmed with the programs of the Conserved Domain Search Service (<http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>). Finally, we identified 19 grape GATA protein sequences, and the sequence data were all downloaded from the Grape Genome Browser (<http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/>).

Phylogenetic analysis, chromosomal location, and gene duplication analyses

Multiple alignments of protein sequences were performed by Muscle in MEGA5 with default parameters. Then, a phylogenetic tree was constructed on the basis of the alignment results by using the neighbor-joining method with 1000 bootstrap replicates. The parameters used in tree construction were Poisson model, uniform rates, and pairwise deletion.

The chromosome map constructed by the Circos software (<http://circos.ca/>) and *VvGATA* gene distribution were determined according to their position information in the Grape Genome Browser (<http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/>). To identify the duplication events of *VvGATA* genes, we analyzed 26,346 protein-coding

genes from the grape genomic database, using the MCScanX software (<http://chibba.pgml.uga.edu/mcscan2/>), The grape protein-coding genes were classified into various types of duplications including tandem duplication and segmental duplication with default parameters (Wang et al. 2012).

Conserved motif, gene structure, and *cis*-element analyses

The protein sequences of VvGATA were analyzed by Multiple Em for Motif Elicitation (MEME) tools (<http://meme-suite.org/tools/meme>) for conserved motif (Bailey et al. 2006). We set the parameters as follows: motif site distribution; any number of repetitions; maximum number of motifs, 15; and minimum and maximum width of the motif, 6 and 80 respectively. Other parameters had default values.

The corresponding genomic DNA sequences and cDNA sequences were obtained from the Grape Genome Browser (<http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/>). Then, the exon and intron structures of *VvGATA* genes were analyzed by Gene Structure Display Server (<http://gsds.cbi.pku.edu.cn/>) (Guo et al. 2007).

The 2000 bp promoter sequences of the 19 *VvGATA* genes were obtained from the Grape Genome Browser (<http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/>).

The *cis*-acting regulatory elements were determined by PLANT CARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) with default parameters.

Plant materials and treatments

Grape seedlings (*V. vinifera* L. cv. Cabernet Sauvignon) and suspension-cultured cells

were used. Cultivar Cabernet Sauvignon is one of the most widely cultivated and economically important fruit crop in the world, and the use of suspension-cultured cells in the study of exogenous treatment offers an advantage in bulk diffusion. Cultivar Cabernet Sauvignon was grown in the grape germplasm resource garden of the Institute of Botany, the Chinese Academy of Sciences, Beijing, under natural conditions. Roots, stems, adult leaves, flowers, and berries at different development stages (fruit setting [F], veraison [V], and ripening [R]) were sampled for gene expression analysis.

Cabernet Sauvignon berry suspension cells were cultured in 250 mL flasks filled with 100 mL of liquid B5 medium (B5 basal medium supplemented with 2.5 g/L hydrolyzed casein, 0.2 mg/L KT, 0.1 mg/L NAA, and 30 g/L sucrose) and centrifuged at 120 rpm in light conditions at 25 ± 1 °C (Zhang et al. 2014). All the suspension cells were subcultured every 7 days. Cabernet Sauvignon berry callus was also cultured in solid B5 medium (liquid B5 medium supplemented with 3 g/L Phytigel) under light conditions at 25 ± 1 °C. The berry callus was subcultured every month. The culture protocol was based on the method described previously with some modifications (Zhang et al. 2014).

Berry callus was grown on solid B5 medium for 2 weeks (logarithmic growth phase of cells), then the callus was subcultured for the light and dark treatments. For the light exclusion treatment, the culture flasks were wrapped in aluminum foil, and the flasks were covered with a shade cloth and placed at 25 ± 1 °C for 2 weeks. For the light-grown treatment, the subculture callus was cultured with light/dark (16:8 h) conditions at $25 \pm$

1 °C. The calluses of the light/dark treatment groups were harvested after two weeks, frozen in liquid nitrogen, and stored at –80 °C for further analysis. For the plant hormone treatment, the suspension-cultured cells were harvested at logarithmic phase (5–6 days after subculture), washed three times with sterile deionized water, and transferred into a 100 mL of hormone-free liquid B5 medium. Then, indole-3-acetic acid (IAA), gibberellin A3 (GA3), abscisic acid (ABA), and 2,4-epicastasterone (EBR) were added into the hormone-free liquid B5 medium to a final concentration of 50 µM. After the hormones were added, the suspension-cultured cells were harvested at 0, 6, and 12 h. The suspension-cultured cells were centrifuged at 5000 rpm for 5 min for the collection of the cells. The collected cells were frozen in liquid nitrogen and stored at –80 °C for further analysis. All the treatments were replicated two or three times.

RNA extraction and real-time PCR analysis

Total RNA was extracted from the different tissues and organs, berry callus, and suspension-cultured cells of Cabernet Sauvignon. An RNAPrep pure plant kit (Qiagen, China) was used for the extraction according to the manufacturer's instructions. For the first-strand cDNA synthesis, the RNA quality was measured by agarose gel electrophoresis, and concentration was detected by a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). First-strand cDNA was synthesized from 500 ng of total RNA with a HiScript Q RT SuperMix for qPCR (+ gDNA wiper) Kit (Vazyme, China). RNA extraction and reverse transcription steps conform to those outlined in the Minimum Information for Publication of Quantitative Real-Time PCR Experiments guidelines

(Bustin, et al. 2009). The qPCR results were completed with the CFX96 System (Bio-Rad, USA), and an AceQ qPCR SYBR Green Master Mix (Vazyme, China) was used. The reaction conditions were as follows: 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. The primer sequences are listed in Table S1. Grape *Actin1* (accession no. AY680701) was used as an internal standard, and the relative expression change was calculated by the formula $2^{-\Delta\Delta C_t}$ (Livak and Schmittgen 2001).

Protoplast isolation and transfection

The whole coding sequence of *VvGATA* was amplified from cDNA from Cabernet Sauvignon berry with gene specific primers (Table S1). The PCR fragment was digested with *EcoR* I and *Kpn* I, then it was subcloned into the pEVS-NL vector for the generation of *pEVS-NL-VvGATA-GFP*, which was driven by the 35S CaMV promoter and contains a *VvGATA-GFP* fusion construct. The construct was confirmed by sequencing. Given that the largescale isolation of viable grape protoplasts is difficult, the tobacco (*Nicotiana benthamiana*) leaf of seed tissues cultured on the MS medium was used for protoplast isolation, and the method used for this step was performed according to the protocol of *A. thaliana* and grapevine mesophyll protoplast isolation (Yoo et al. 2007; Zhao et al. 2016). Protoplasts were transfected through a polyethylene glycol method described previously (Zhao et al. 2016). Transfected protoplasts were incubated for 12–16 h at 25 °C in the dark. The fluorescence signals were analyzed by confocal microscopy (Leica TCS SP5, Germany). GFP fluorescent signals were excited at 488 nm, and auto-fluorescence was captured at 505–535 nm. The transient expression experiment was repeated at least three

211 times.

212 **Results**

213 **Identification of the GATA factor family in grape**

214 For the identification of potential *GATA* genes in grape, a BLAST program was carried
215 out by using the 29 *Arabidopsis* GATA proteins against the grape genome sequence
216 (Reyes et al. 2004; Manfield et al. 2007). We constructed a hidden Markov model to
217 search for the GATA sequences. All these putative sequences were analyzed in the
218 conserved GATA zinc finger domain at the NCBI Conserved Domain Search Service. A
219 total of 19 *GATA* genes were identified. This result was consistent with that obtained in
220 the Plant Transcription Factor Database (PlantTFDB, <http://planttfdb.cbi.pku.edu.cn/>). A
221 summary of the gene ID, conserved domain accession number, and short name of the 19
222 *GATA* genes are listed in Table S2.

223 We named these 19 *GATA* genes according to phylogenetic analysis results and
224 those that best matched the *Arabidopsis* GATA proteins (Table 1 and Fig. 1). *VvGATA*-
225 encoded peptides ranged from 109 to 386 amino acids and were classified into four
226 subfamilies (Table 1). These GATA genes were distributed on 11 grape chromosomes,
227 which displayed an unbalanced distribution. Four *VvGATA* (*VvGATA2*, *VvGATA5*,
228 *VvGATA9*, *VvGATA10*) genes were mapped on chromosome 4; three *VvGATA*
229 (*VvGATA3*, *VvGATA16*, and *VvGATA18*) genes on chromosome 3; two on chromosomes
230 9, 14, and 18; and only one gene on chromosomes 5, 6, 8, 11, 12 and 15 (Fig. 2).
231 *VvGATA15* and *VvGATA17* were separated by only 1.8 kb on chromosome 18.

Phylogenetic relationships, conserved motifs, and gene structures of GATA genes in grape

To investigate the phylogenetic relationships of *GATA* genes, we constructed a phylogenetic tree, using the GATA protein sequences from grape and *Arabidopsis* (Fig. 1). The result revealed that 19 *VvGATA* genes were divided into four subgroups (I, II, III, and IV), which had 7, 6, 5, and 1 *VvGATA* members, respectively. The grape GATA family is contracted in contrast to that of *Arabidopsis*. Three subgroups (I, II and IV) are contracted in grape. Each subgroup had one or more pairs of GATA proteins with a high bootstrap value. This finding suggested that the *VvGATA* genes in each subgroup have a common origin. Moreover, the GATA genes from the same lineage tended to cluster together in the phylogenetic tree. This suggested that they experienced duplications after the divergence of grape and *Arabidopsis*. And no species-specific subgroups were observed, implying that GATA genes were more conserved during evolution. The functions of some *AtGATA* genes are well characterized in *Arabidopsis*; therefore, the evolutionary relationship of *GATA* genes between grape and *Arabidopsis* may provide insights into their roles in grape development.

Gene duplication events play an important role in the evolution of a gene family, and tandem and segmental duplications are the two main mechanisms (Cannon et al. 2004). To detect the potential evolutionary relationships among *VvGATA* genes, we analyzed tandem duplication and segmental duplication events within the grape genome. The result indicated that 17 *VvGATA* genes underwent segmental duplications and one

pair of genes (*VvGATA15* and *VvGATA17*) was assigned to a tandem duplication (Fig. 2), suggesting that segmental duplication events have played a major role in the expansion of the *VvGATA* gene family.

Conserved motifs and gene structures play important roles in multiple gene families, and revealing the diversity of motifs and gene structures may provide an explanation for the evolution of gene families. To reveal the diversification of *VvGATA* genes in grape, we analyzed the conserved motifs. The analysis was performed on a MEME program. Ten distinct conserved motifs were identified in 19 *VvGATA* proteins (Fig. 3a). All the grape *GATA* genes contained a GATA zinc finger. *VvGATA14*, *VvGATA15*, *VvGATA16*, and *VvGATA17* contained a CCT motif (pfam06203) and TIFY domain (pfam06203) apart from a GATA zinc-finger domain (Table S2).

To verify the structure of the *VvGATA* genes, we analyzed the exon and intron boundaries. The results showed that genes in the same subgroup generally had the same exon/intron number, position, or length (Fig. 3b). For example, *VvGATA14*, *VvGATA15*, *VvGATA16*, and *VvGATA17* showed similar gene structure patterns. Different subgroups had different in gene structure. For instance, most members of subfamily I contain three exons. The genes of subgroup III contain seven, eight or eleven exons, and these genes had long introns. The variation in the structures of grape *GATA* gene family indicates that the *VvGATA* genes changed during their evolutionary history. This change might have led to the considerable functional diversity in the *VvGATA* gene family.

***cis*-Elements in the promoter region of *VvGATA* genes**

274 To verify the *cis*-elements involved in the regulation of *VvGATA* genes, we obtained
275 2000 bp DNA sequences upstream of the *VvGATA* genes and identified *cis*-acting
276 regulatory elements. PLANT CARE
277 (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>) was used for the
278 identification. We identified 99 *cis*-elements, and most of them were classified as light-
279 responsive elements (Table S3). Some *cis*-elements were highly conserved in the
280 promoter region of the *VvGATA* genes, and 17 *cis*-acting regulatory elements were
281 ubiquitous among the *VvGATA* gene members (Table 2). The *cis*-elements were
282 responsive to light, circadian rhythm, plant hormones, and abiotic or biotic stresses. For
283 instance, two light-responsive elements (Box 4, Box I), a heat-stress element (HSE), and
284 an endosperm expression element (Skn-1_motif) were represented in nearly all the
285 *VvGATA* genes. Plant hormones, such as abscisic acid, salicylic acid, gibberellin, MeJA,
286 ethylene, and auxin, also had a responsive motif in the promoter region of the *VvGATA*
287 members (Table 2, Table S3).

288 **Expression profiles of *VvGATA* genes in different grape tissues**

289 We analyzed the expression pattern of the *VvGATA* genes in the roots, stems, adult
290 leaves, flowers, and berries of grape (*V. vinifera* L. cv. Cabernet Sauvignon) at fruit
291 setting [F], veraison [V], and ripening [R] (Fig. 4). Quantitative RT-PCR results showed
292 that all *VvGATA* genes were expressed, except *VvGATA4* and *VvGATA19*, and most of
293 the *VvGATA* genes displayed higher expression levels in vegetative organs than in berry
294 tissues (Fig. 4). Some *VvGATA* genes showed minimal variation among organs and

tissues (Fig. 4a), and other *VvGATA* genes displayed wider variation (Fig. 4b). For instance, *VvGATA1*, *VvGATA3*, *VvGATA6*, *VvGATA10*, and *VvGATA11*, were highly expressed in the leaves, *VvGATA5* and *VvGATA7* in the flowers, and *VvGATA3* in the roots. Notably, *VvGATA11* displayed the highest expression level in the adult leaves. Overall, most of *VvGATA* genes are highly expressed in vegetative organs. The expression patterns in vegetative organs indicate that *VvGATA* genes have important roles in source tissues.

RNA-seq data are useful resources for studying gene transcript abundance. To obtain insight into the transcript patterns of the *VvGATA* genes during fruit development, we analyzed the three developmental stages of berry (fruit setting [F], veraison [V], and ripening [R]) of Cabernet Sauvignon RNA-seq data from our unpublished database (Fig. 4c). The *VvGATA* genes were divided into four main clades, and the transcript abundances of *VvGATA9*, *VvGATA11*, and *VvGATA19* were not detected in the berries. Clade 1 contained four genes. *VvGATA17* was highly expressed in all stages, whereas the other genes displayed preferential expression in some stages. *VvGATA16* in clade 2 exhibited the highest stable expression in the berry tissues. The genes in clade 3 exhibited high expression levels in the fruit setting stage. By contrast, the genes in clade 4 were lowly expressed during berry development.

Subcellular localization of the VvGATA proteins

Generally, grape GATA proteins contain a conserved GATA zinc-finger domain. However, in addition to *VvGATA18*, subgroup III GATA proteins also contain two other

conserved domains (CCT, TIFY). To determine VvGATA subcellular localization, we selected VvGATA14, VvGATA15 and VvGATA18 as reference proteins for nuclear targeting. The entire coding sequence of *VvGATA* from *V. vinifera* L. cv. Cabernet Sauvignon was fused in frame to the GFP coding sequence under the control of the CaMV 35S promoter, and the recombinant constructs were transfected into tobacco protoplasts. As shown in Fig. 5, the pEVS-NL-VvGATA14-GFP and pEVS-NL-VvGATA15-GFP fusion proteins predominantly accumulated in the nucleus, whereas the pEVS-NL-VvGATA18-GFP was targeted to the nucleus, although the accumulation of the fusion protein was not restricted to the nucleus. The VvGATA18 protein was also located at the extranuclear compartment. These results indicate that nuclear targeting of the VvGATA protein is consistent with their predicted transcriptional regulatory function.

Expression patterns of *VvGATA* genes in response to light and phytohormones

We investigated *VvGATA* gene functions by using heterotrophic-cultured cells obtained from grape berries as a model system. We focused on grape GATA transcription factors that play important roles in berry development. Our quantitative RT-PCR results and RNA-seq data revealed that *VvGATA* genes in subgroup III (*VvGATA14*, *VvGATA15*, *VvGATA16*, *VvGATA17*, *VvGATA18*) were more highly expressed than those in the other subgroups in berries. Thus, we assayed the expression levels of grape GATA subgroup III genes under light/dark treatment. As shown in Fig. 6a, the selected *VvGATA* genes (subgroup III) were all up-regulated by light, and the transcript abundance of *VvGATA18*

showed twofold increase under light treatment. *VvGATA14* and *VvGATA16* showed 1.5-fold difference between the light-grown and dark-grown berry callus, whereas the others showed only a slight difference. These results indicated that the *VvGATA* genes are involved in light-responsive regulation.

Phytohormones play an important role in the berry development of grape. To determine whether *VvGATA* genes are responsive to hormone signaling, we analyzed the expression level of the selected *VvGATA* genes (subgroup III) by using exogenous indole-3-acetic acid (IAA), gibberellin A3 (GA3), abscisic acid (ABA), and 2,4-epicastasterone (EBR) treatments. The expression of *VvGATA14* and *VvGATA16* displayed continuous up-regulation for 12 h, whereas the other genes showed slight variation under exogenous IAA treatment (Fig. 6b). *VvGATA14* transcript accumulation increased after the GA3 and ABA treatments (Fig. 6c, d), whereas the transcript accumulation of *VvGATA17* or *VvGATA18* was down-regulated under the GA3 treatment (Fig. 6c). Additionally, all the selected *VvGATA* genes showed up-regulation after EBR treatment (Fig. 6e).

Interestingly, *VvGATA14* displayed response to all these phytohormones. These data suggest that *VvGATA* genes might also be involved in phytohormone signaling pathway.

Discussion

GATA transcription factors are important to plant development. In this study, we showed a fundamental exploration and analysis of the grape GATA gene family, and provided insight into the potential functions of the *VvGATA* genes in light and phytohormone response during grape berry development. To identify the grape GATA transcription

factors, we performed a genome-wide scan of the grapevine genome sequence (Jaillon et al. 2007). A total of 19 *VvGATA* protein-encoding genes were identified. Meanwhile, a phylogenetic tree was constructed to predict the evolutionary relationships of *VvGATA* genes and the potential functions based on homologous genes (*AtGATA2*, *GNC*, *GNL*, and *HAN*) that have been well studied in *Arabidopsis*. The grape GATA family was classified into four major subfamilies (I, II, III, and IV; Fig. 1). However, subgroup III was substantially expanded in grape. *Arabidopsis* subgroup III genes were found to contain a CX₂CX₂₀CX₂C motif, exclusively conserved domain CCT, and TIFY (ZIM), which belong to a novel plant-specific GATA-type transcription factors (Shikata et al. 2004; Vanholme et al. 2007). *VvGATA* proteins in subgroup III also contain this domain (*VvGATA14*, *VvGATA15*, *VvGATA16*, and *VvGATA17*). ZIM proteins or TIFY proteins are involved in petiole and hypocotyl elongation, flower development, or root development in *Arabidopsis* (Nishii et al. 2000; Shikata et al. 2004; Vanholme et al. 2007), and our expression pattern analysis showed that *VvGATA* genes in subgroup III were more highly expressed during berry developmental stages than the other *VvGATA* genes. This result indicated that *VvGATA* genes in subgroup III might be involved in sink organ developmental processes. B-GATAs, also known as *Arabidopsis* subgroup II genes were identified to play a key role in plant growth and development, such as in greening and flowering (Behringer and Schwechheimer 2015). Considering these B-GATA genes, *GNC*, *CGA1/GNL*, and *HAN*, were well-characterized, the grape subgroup II homologous genes might be candidate genes for further study.

379 The expression profiles of the *VvGATA* genes in different grape organs were
380 investigated. Our results revealed that most of the *VvGATA* genes are highly expressed in
381 leaves, stems, and flowers. The results also support the idea in previous reports that the
382 GATA factors are involved in light response, chloroplast biogenesis, and floral
383 development (Behringer et al. 2014; Behringer and Schwechheimer 2015). According to
384 our qRT-PCR analysis results, seven genes in subgroup I exhibited multiple expression
385 profiles in different grape tissues. *VvGATA1* and *VvGATA6* are close members in grape
386 subgroup I, and both of them showed high expression level in the leaves. Furthermore,
387 many light-responsive elements were identified in the promoter regions of *VvGATA1* and
388 *VvGATA6*. These results implied the potential roles of these genes in light-regulated
389 development. *VvGATA2*, *VvGATA5*, and *VvGATA7* were highly expressed in the flowers,
390 and the transcript abundance of *VvGATA5* was higher in berry ripening stage than in
391 vegetative organs (Fig. 4), implying that *VvGATA2*, *VvGATA5*, and *VvGATA7* likely
392 played a role in the sink tissues. In *Arabidopsis*, *AtGATA2* is a positive regulator of
393 photomorphogenesis, which is highly expressed in hypocotyls and petioles and is a key
394 light-signaling transcriptional regulator that mediates the crosstalk between
395 brassinosteroid (BR) and light-signaling pathways (Luo et al. 2010). *AtGATA2* and
396 *AtGATA4* are the two closest members in *Arabidopsis* subfamily I, the homologous gene
397 to these genes in grape was *VvGATA4*, the expression of which is barely detectable in
398 different grape organs according to our qRT-PCR data. Our RNA-seq data showed that
399 *VvGATA4* is highly expressed in the fruit setting stage during berry development.

Therefore, further study of the functional difference among plant species is necessary. In grape subgroup II, *VvGATA8* was highly expressed in grape organs, especially in the stem, leaf, and flower. The orthologous genes of *VvGATA8* in *Arabidopsis* are *AtGATA15* and *AtGATA16*, which participate in greening, hypocotyl elongation, phyllotaxy, floral organ initiation, accessory meristem formation, flowering time, and senescence (Ranftl et al. 2016). Likewise, the orthologous gene in rice, *OsGATA12*, regulates chlorophyll levels, senescence, and yield parameters (Lu et al. 2017). These results suggest that *VvGATA8* plays an important role in grape growth and development. Thus, *VvGATA8* should be considered a candidate gene for developmental responses in grape. The GATA factor HAN (*AtGATA18*) mediates embryogenesis and flower development in *Arabidopsis* (Zhao et al. 2004; Nawy et al. 2010; Zhang et al. 2013). However, the homologous gene *VvGATA9* displayed low expression levels in different grape organs and tissues. *VvGATA10* and *VvGATA11* displayed tissue-specific expression; in particular, *VvGATA11* displayed high-level of expression in leaves. *GNC*, a homologous gene of *VvGATA10* and *VvGATA11*, exhibits multiple biological functions in higher plants, particularly in germination, greening, chlorophyll synthesis, flowering time, senescence, and glucose sensitivity (Bi et al. 2005; Chiang et al. 2012; Behringer et al. 2014; Behringer and Schwechheimer 2015; Ranftl et al. 2016; Klermund et al. 2016). These results provide an insight into the potential function of *VvGATA10* and *VvGATA11* in regulating carbon metabolism and other biological processes.

In grape berry tissues, some *VvGATA* genes in subgroup III displayed higher

expression than the other *VvGATA* genes according to our RNA-seq data (Fig. 4c). *VvGATA14* displayed higher expression before veraison, *VvGATA15* was mainly expressed in fruit setting and ripening stages, and *VvGATA16* and *VvGATA17* were highly expressed in all berry developmental stages. Thus, *VvGATA14*, *VvGATA15*, *VvGATA16*, and *VvGATA17* should be candidate genes for further study in berry development. The subcellular localization of *VvGATA14*, *VvGATA15*, and *VvGATA18* displayed the nuclear localization of *VvGATA*, and this result is consistent with the regulatory function of *VvGATA* as a transcription factor. Many studies demonstrated that light or phytohormones play important roles in grape growth and berry development (Deluc et al. 2007; Kuhn et al. 2013; Böttcher et al. 2013; Kondo et al. 2014; Xu et al. 2015) and some GATA genes are responsive to light or hormone signals (Luo et al. 2010; Richter et al. 2013). In this study, *VvGATA18* and *VvGATA14* were significantly up-regulated under light treatment and exogenous phytohormone treatment, respectively.

In this study, we provided basic information about *VvGATA* genes. Nevertheless, the details of the molecular regulation mechanism of the *VvGATA* transcription factors in grape still needs further study.

Author contribution statement ZCL, SHL and ZZ designed the experiments. ZZ performed the experiments, analyzed results and manuscript preparation. CR, LMZ and YW performed bioinformatics analyses. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Draft

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Figure legends

Fig. 1 Phylogenetic analysis of GATA proteins. The amino acid sequences from grape and Arabidopsis were used to construct a tree in MEGA 5 using the neighbor-joining method with 1000 bootstrap replicates. The parameters used in the tree construction were poisson model, uniform rates and pairwise deletion. The four clades are marked with different colors.

Fig. 2 Chromosomal locations and gene duplication of grape *GATA* genes. The chromosomal units are in 100,000 bases. The chromosome number is shown inside the circle. The tandem duplication and WGD/segmental duplication genes are connected by a blue curve and red curves, respectively.

Fig. 3 Gene structures and motif composition of grape GATA factors. a. Schematic diagram of conserved motifs in VvGATA proteins. Each colored box represents a

673 conserved motif in the protein, the black lines represent the non-conserved sequences. b.
674 Exon/intron structures of the *VvGATA* genes. Yellow boxes represent exons, spaces
675 between the boxes correspond to introns, and blue boxes represent untranslated region.
676 The size of genes can be estimated using the horizontal scale.

677 **Fig. 4** Expression profiles of the *VvGATA* genes in grape organs (*V. vinifera* L. cv.
678 Cabernet Sauvignon). Expression was analyzed by real-time PCR using grape *Actin1* as
679 reference gene. The gene expression level in different organs showing the highest CT
680 values was set to 1. Data are presented in two separate graphs, a and b; genes in b display
681 the highest relative expression levels. Data are presented as mean $\pm$ SD. There were two
682 biological replicates and the expression level of each was calculated from the mean value
683 of three technical replicates. c. Expression patterns of *VvGATA* genes in different berry
684 development stages from the RNA sequence; the berry samples of *V. vinifera* L. cv.
685 Cabernet Sauvignon were collected at fruit set (F), veraison (V), and ripening (R). The
686 color scale represents transcript abundance which was the normalized FPKM values.
687 Green indicates low levels, black indicates a medium level and red indicates high levels
688 of transcript abundance.

689 **Fig. 5** Subcellular localization of VvGATA proteins. The control 35S::GFP construct,
690 VvGATA14-GFP, VvGATA15-GFP, and VvGATA18-GFP fusion proteins were
691 transiently expressed in tobacco (*N. benthamiana*) protoplasts. The GFP green
692 fluorescence, chlorophyll autofluorescence, and bright-field images are shown (scale bar:
693 10 μ m).

Fig. 6 Expression patterns of subgroup III GATA genes in response to light and phytohormones. a. Expression patterns of subgroup III genes under light/dark treatment. b. IAA-induced expression patterns of subgroup III genes. c. GA3-induced expression patterns of subgroup III genes. d. ABA-induced expression patterns of subgroup III genes. e. EBR-induced expression patterns of subgroup III genes. The transcript levels were analyzed by real-time PCR. Data are presented as mean $\pm$ SD. There were two biological replicates and the expression level of each was calculated from the mean value of three technical replicates.

Table legends

Table 1 GATA genes in grape

Table 2 Common putative *cis*-elements in the promoter of the *VvGATA* genes

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- Captions for Supplementary tables**
- Table S1** Primers used in this study
- Table S2** GATA transcription factors in grape
- Table S3** *cis*-elements in the promoter of the *VvGATA* genes

Table 1 GATA genes in grape

Gene name	GenBank accession no.	Gene ID	Genome location	Peptide length	Sub family
<i>VvGATA1</i>	XR_786007	GSVIVT01025199001	chr6:3427975..3432516	386	I
<i>VvGATA2</i>	XM_002283709	GSVIVT01035614001	chr4:2730001..2731754	196	I
<i>VvGATA3</i>	XM_002274836	GSVIVT01024194001	chr3:452476..454201	352	I
<i>VvGATA4</i>	XM_002277923	GSVIVT01018180001	chr15:13505485..13506854	255	I
<i>VvGATA5</i>	XM_002272726	GSVIVT01018833001	chr4:19467932..19469492	305	I
<i>VvGATA6</i>	XM_002282189	GSVIVT01033385001	chr8:21037307..21039075	265	I
<i>VvGATA7</i>	XM_002283992	GSVIVT01017011001	chr9:3439047..3440507	283	I
<i>VvGATA8</i>	XM_010651243	GSVIVT01035048001	chr5:1163455..1164868	153	II
<i>VvGATA9</i>	XM_010650837	GSVIVT01018951001	chr4:18421693..18423027	285	II
<i>VvGATA10</i>	XM_002279247	GSVIVT01035406001	chr4:1062805..1064236	306	II
<i>VvGATA11</i>	XM_002282137	GSVIVT01015204001	chr11:1817933..1819418	309	II
<i>VvGATA12</i>	XM_010662532	GSVIVT01032362001	chr14:26715231..26732295	367	II
<i>VvGATA13</i>	XM_002278333	GSVIVT01031203001	chr14:1203196..1204273	109	II
<i>VvGATA14</i>	XM_010657000	GSVIVT01029593001	chr9:20973735..20988914	244	III
<i>VvGATA15</i>	XM_002283717	GSVIVT01009197001	chr18:6040226..6058566	248	III
<i>VvGATA16</i>	XM_002270325	GSVIVT01012518001	chr3_random:451954..465083	254	III
<i>VvGATA17</i>	XM_010666122	GSVIVT01009198001	chr18:6060381..6094675	324	III
<i>VvGATA18</i>	XM_010646869	GSVIVT01012523001	chr3_random:527226..560073	188	III
<i>VvGATA19</i>	XM_019223388	GSVIVT01013319001	chr12:11878739..11880214	259	IV

Table 2 Common putative *cis*-elements in the promoter of the *VvGATA* genes

<i>cis</i> -Element name	Sequence	Response	Number
Box 4	ATTAAT	Light	16
Box I	TTTCAAA	Light	16
I-box	GATANGG	Light	12
TCT-motif	TCTTAC	Light	12
G-box	CACGTG	Light	14
GA-motif	AAAGATGA	Light	10
GAG-motif	RGAGAKK	Light	10
Sp1	CCRCCC	Light	12
Circadian	CAANNNNATC	Circadian	13
ABRE	CACGTG	Abscisic acid	10
TCA-element	TCATCTTTTT	Salicylic acid	10
MBS	YAACTG	Drought	12
TC-rich repeats	TTTTCTT	Defense and stress	14
HSE	AAAAAATTYS	Heat stress	16
ARE	TGGTTT	Anaerobic induction	13
Skn-1_motif	GTCAT	Endosperm	16
5UTR Py-rich stretch	TTTCTTCTCT	Conferring high transcription levels	11

Number, a frequency of each *cis*-element appeared in 19 *VvGATA* gene promoters.

Figure 1 Phylogenetic analysis of GATA proteins.

223x223mm (300 x 300 DPI)

Figure 2 Chromosomal locations and gene duplication of grape GATA genes.

181x164mm (300 x 300 DPI)

Figure 3 Gene structures and motif composition of grape GATA factors

221x73mm (300 x 300 DPI)

Figure 4 Expression profiles of the VvGATA genes in grape organs
254x76mm (300 x 300 DPI)

Figure 5 Subcellular localization of VvGATA proteins.

142x178mm (300 x 300 DPI)

Figure 6 Expression patterns of subgroup III GATA genes in response to light and phytohormones

181x87mm (300 x 300 DPI)