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Review

Isolation and analysis of differentially expressed genes during ovule abortion in the seedless grape

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ABSTRACT

The propagation of seedless grapes is currently one of the most important global trends in agricultural production and consumption. However, stenospermocarpy has not been characterized at the molecular level to date. In the present study, cDNA sequence-related amplified polymorphism (SRAP) analysis using 17 SRAP primers was utilized to identify differentially expressed genes during ovule development in *Vitis vinifera* L. cv. Youngle and *V. Vinifera* L. cv. Pinot noir. A total of 24 ESTs were detected, with lengths ranging from 147 bp to 1785 bp. These ESTs were deposited to GenBank (Accession numbers JK818295–JK818319). EST differential expression analysis was performed in two grape species, followed by cloning, from which 8 genes were selected for expression profiling in different tissues during ovule development of *V. vinifera* L.cv. Youngle and *V. Vinifera* L. cv. Pinot noir. The genes YG7931, YG38221, and YG5571 showed stage-specific expression during ovule development.

Contents

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1. Introduction

Superior adaptability, early fruit maturity, higher fruit number per plant, good taste, and high nutritive value are major characteristics that have made grapes a popular food item in the human diet. With the rapid development of the grape industry, the demand for seedless grapes has significantly increased in the world market. To date, the development of the seedless grapes is one of principal objectives of grape breeders around the world. Therefore, research on the mechanism underlying the production of seedless grape is of particular importance. Two mechanisms that are involved in the production of seedless grapes include parthenocarpy and stenospermocarpy (Cabezas et al., 2006; Ingrosso et al., 2011; Sarikhani et al., 2009; Stout, 1936). However, most commercial seedless grapes are generated via stenospermocarpy, which involves pollination and fertilization, while the ovule development is aborted. Therefore, stenospermocarpy cultivars are not strictly seedless but contain seminal rudiments or traces of seeds of different sizes (Ledbetter and Ramming, 2011). *V. vinifera* L. cv. Youngle is a stenospermocarpy-generated seedless grape that underwent natural pollination; this cultivar consists of early-maturity varieties. In Yangling, Shaanxi, China, its full bloom stage is in late May, and its fruits mature in mid- to late July.

Although the production of seedless grapes has been performed for more than a decade, but the genetics of seedless grape are complex and not yet fully understood. Various theoretical models have been presented via traditional genetic methods; However, no common view has been established to date. The four important models of seedless grapes production are as follows: 1. quantitative trait loci (QTLs) model (Cabezas et al., 2006; Doligez et al., 2002; Fischer et al., 2004; Liu and Clingeleffer, 2008); 2. dominant model (Ledbettter and Burgos, 1994; Roytchev, 1998); 3. recessive genetic model (Dudley, 1993); and 4. A complex system (Notsuka et al., 2001).

Research studies on the mechanism of grape ovule abortion at the genetic level are limited. A Suppression subtractive hybridization (SSH) cDNA library was prepared by Hanania, and the chloroplast chaperonin 21 (ch-Cpn21) gene was screened in two 'Thompson' lines, a seeded and a seedless, and silencing of ch-Cpn21 in Nicotiana benthamiana plants and Lycopersicum esculentum fruits resulted in seed abortion. These results suggested that ch-Cpn21 plays an important role in stenospermocarpic grape (Hanania et al., 2007). Costenaro-da-Silva developed a modified representational difference analysis (RDA) method named bulk representational analysis of transcripts (BRAT) to identify genes specifically associated with each of the main developmental stages of sultanine grapevine berries (Costenaro-da-Silva et al., 2010). Mejia et al. (2011) identified VvAGL11 with several SNPs and INDELs in both regulatory and coding regions. These INDELs and SNPs respectively explained up to 44% and 78% of the phenotypic variation in seed and berry weight. Moreover, no expression of the VvAGL11 in seedless genotypes was detected during seed development. Wang identified an EF-hand calcium-binding protein, VvCBP, which was differentially expressed in the embryo of the seedless and seeded grapevine species. Moreover, silencing the homologous gene in tomato resulted in a significant decrease in seed number (Wang et al., 2011). These findings suggested that the VvCBP gene is also involved in ovule abortion. In addition, Wang et al. (2011) identified 54 grape MADS-box genes after treating the plants with gibberellic acid (GA3), as well as screened a few candidate genes such as VvMADS28, VvMADS39, VvMADS44, VvMADS45, and VvMADS46, which showed differential expression during ovule development in seeded and seedless cultivars.

Sequence-related amplified polymorphism (SRAP) is a PCR-based molecular marker technique developed by Li and Quiros (2001). It involves RNA extraction, cDNA synthesis, PCR amplification, and electrophoresis. It is a less expensive and simpler tool for the analysis of differential gene expression patterns in various plant species. SRAP was used to identify a salt-responsive gene in English cordgrass roots that showed 30% similarity to theβ-1,3-glucanase of rice (Lu and Wu, 2006). Deng utilized cDNA-SRAP to study the differential gene expression of the restorer and maintainer lines of cabbage. Amplification using 30 SRAP primer combinations identified two differentially expressed genes (Deng et al., 2007).

With the completion of the grape genome sequence, research on novel genomic techniques entered a new stage. However, stenospermocarpy has not yet been characterized at the molecular level. To study the mechanism underlying the development of seedless grapes, molecular markers were used to search for the grape ovule abortion-related gene. In the present study, SRAP-cDNA analysis was performed to identify differentially expressed genes during ovule development in *V. vinifera* cv. Youngle and cv. Pinot noir. The differentially expressed ESTs of two grape species were analyzed and cloned, from which 8 genes were selected for expression profiling in different tissues during ovule development in *V. vinifera* L. cv. Youngle and *V. vinifera* L. cv. Pinot noir. These results provide new hypotheses that may be examined in future studies on the mechanism underlying the generation of seedless grapes.

2. Materials and methods

2.1. Plant materials and sampling conditions

Vitis vinifera L. cv. Youngle and cv. Pinot noir were cultivated in the Grape Repository of Northwest A&F University, Yangling, Shanxi, China under a natural environment and normal management. The sampling method performed was as previously described (Wang et al., 2011). To maintain consistency in flowering, the blooming flowers and ear tip buds were removed from the grape inflorescences prior to the full-bloom stages, and at the full-bloom stages, any unopened alabastrum were removed and then covered with a bag. After the full-bloom stages of 10 d, 15 d, 20 d, 25 d, 30 d, 35 d, 40 d, and 45 d, the embryos of *V. vinifera* L. cv. Youngle and V. vinifera L. cv. Pinot noir were collected, and the ovules were stripped out and placed in centrifuge tubes on ice. Subsequently, the tubes were flash-frozen in liquid nitrogen prior to storage in −80 °C. The root, stem, leaf, alabastrum, and tendril were collected from the same plant of *V. vinifera* L. cv. Youngle on May 18, the flowers were collected on May 22, and the pericarp and pulp on June 27. These tissues were also flash-frozen in liquid nitrogen and stored at −80 °C.

Using the improved SDS/phenol method (Zhang et al., 2003), total RNA was extracted from the various ovule stages of *V. vinifera* L. cv. Pinot noir and cv. Youngle, as well as from the root, stem, leaf, Tendril, alabastrum, floral, pericarp, pulp of *V. vinifera* L. cv. Youngle. RNA quality was evaluated by agarose gel electrophoresis and spectrophotometry. Reverse transcription was performed with 2.0 μg of total RNA after DNase digestion, using RevertAid First Strand cDNA Synthesis Kit (Fermentas).

2.2. SRAP analysis

SRAP was conducted as previously described (Li and Quiros et al., 2001), with minor modifications. RT-PCR was conducted using the primer pairs for SRAP analysis (Table 1). The cDNA from the ovules of *V. vinifera* L. cv. Pinot noir and cv. Youngle in eight developmental stages was used as template for cloning. PCR reactions were performed in total volume of 25.0 L and the conditions for RT-PCR were as follow: 2.0 L of the dNTP mix (2.5 mM), 2.5 L of the LA PCR buffer (10 ×), 0.2 L of LA Taq (5U/L), 1.0 L of cDNA, 1.0 L each of the forward

Table 1
SRAP primers used in this study for RT-PCR.

Forward primers	Primer sequence	Reverse primers	Primer sequence
Me1	TGAGTCCAAACCGGATA	Em1	GACTGCGTACGAATTAAT
Me2	TGAGTCCAAACCGGAGC	Em2	GACTGCGTACGAATTTGC
Me3	TGAGTCCAAACCGGAAT	Em3	GACTGCGTACGAATTGAC
Me4	TGAGTCCAAACCGGACC	Em4	GACTGCGTACGAATTTGA
Me5	TGAGTCCAAACCGGAAG	Em5	GACTGCGTACGAATTAAC
Me6	TGAGTCCAAACCGGTAA	Em6	GACTGCGTACGAATTGCA
Me7	TGAGTCCAAACCGGTCC	Em7	GACTGCGTACGAATTGAG
Me8	TGAGTCCAAACCGGTGC	Em8	GACTGCGTACGAATTGCC
		Em9	GACTGCGTACGAATTTCA

Table 2
qRT-PCR primers of 8 candidate genes used in this study.

Primer	Primer sequence	Primer	Primer sequence
YG1461-F	GGTTATGGTTTGATAGGAATCTTAGG	YG1461-R	CCACATTTATTGCCAACTCTCTG
YG1921-F	GCCAACCAGGCTCTTCTTTCT	YG1921-R	CCAGCATCACTCAGGCACTTC
YG38122-F	ATTGATTGGGCTTGGTTATGG	YG38122-R	ATGAGCTTGTTTCTCCGCTTC
YG2621-F	AGCCAAAGCAGCAATGTAATG	YG2621-R	AGATAAGGCGGCAGAAGGA
YG2951-F	TTCCTCATCGGAGCATTTGT	YG2951-R	CAATGAGTTGGATTTGGGTGG
YG5571-F	TGCTAAGCAAGGCAGGGTG	YG5571-R	CACGCACTGTTCAGATTTGTAAG
YG2973-F	CTCTACCCTACCCAAGACCCA	YG2973-R	TCGTAGAACAAAGCCTCATCCT
YG7931-F	TCCTCTAATCCATACCCCCAA	YG7931-R	GCTGCGGTTAACAAGACAGTG
ACT-F	CTCTATATGCCAGTGGGCGTAC	ACT-R	CTGAGGAGCTGCTCTTTGCAG

and reverse primers, and 17.5 L of ddH2O. The SRAP markers were amplified using the following parameters: 95 °C for 5 min, 94 °C for 30 s, 50 °C for 30 s, and 72 °C 2.5 min for 5 cycles; 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 2.5 min for 35 cycles, and 72 °C for 10 min. The PCR products were detected by 1% agarose gel electrophoresis. Genes of the same length but differentially expressed at different periods were detected in the two grapes. The PCR products of the *V. vinifera* L. cv. Youngle were ligated with the Pgem-T-easy vector (Takara) and then transformed into Escherichia coli TOP 10. Finally, the products were sequenced after PCR screening of bacterial colonies. Cloning of each gene was performed in 3 replicates, following by mapping the location of the corresponding gene in the grape genome. Finally, a total of 24 ESTs were detected by cDNA-SRAP. Further analysis indicated that the homologous genes of the candidate gene were involved in ovule development. Combined with the result of the SRAP, the eight genes were selected and further assessed by quantitative RT-PCR using the primer pairs presented in Table 2.

2.3. Expression profiling of candidate genes in different tissues

The cDNA of the root, stem, leaf, Tendril, alabastrum, floral, pericarp, pulp of the *V. vinifera* L. cv. Youngle were generated. The PCR reactions were conducted using a total volume of 25.0 L, and using the following conditions: 2.0 L of the dNTP mix (2.5 mM), 2.5 L of the LA PCR buffer (10×), 0.2 L of the LA Taq (5U/L), 1.0 L of the cDNA, 1.0 L each of the forward and reverse primers, and 17.5 L of ddH2O. Actin was amplified using the following conditions: 94 °C for 1 min, 94 °C for 30 s, 55 °C for 1 min, 72 °C for 2 min for 28 cycles, followed by 72 °C for 5 min. Eight candidate genes were amplified as follows: 94 °C for 1 min, 94 °C for 30 s, 50 °C for 1 min, and 72 °C for 2 min for 32 cycles, followed by 72 °C for 5 min. The PCR products were detected on 1.5% agarose gel electrophoresis.

2.4. qRT-PCR analysis of transcript levels

Total RNA and first-strand cDNAs were prepared. qRT-PCR was conducted using the primer pairs of the candidate genes, and the actin were used as internal controls (Table 2). Each primer pair was designed to attach to the 3′-terminal of the target sequence. The reactions employed SYBR Green I (TaKaRa) and were conducted using an iQ5 real-time PCR system (Bio-Rad Laboratories, USA), the reaction conditions were as follows: 1 μL of the cDNA, 1 μL of 10 μM primer-F, 1 μL of 10 μM primer-R, 12.5 μL of SYBR Premix Ex TaqTMII (2×), and ddH_2O to a final volume of 25 μL. The thermal parameters of qRT-PCR were as follows: 95 °C for 3 min, followed by 45 cycles of 95 °C for 5 s and 60 °C for 30 s, and a final extension at 72 °C for 30 min. The grapevine actin gene was used as internal control. The relative expression levels of each candidate genes were analyzed using the software, iQ5.

3. Results

3.1. Identification of differentially expressed genes in stenospermocarpic seedless grapes

Seventeen primers (Table 1) were used in RT-PCR amplification. The PCR products were analyzed by agarose gel electrophoresis, and differentially expressed genes were identified as those showing the same length fragment but different brightness (Fig. 1). The products amplified using the Me1 Em4 primers were 600 bp in length. The expression of this gene continued to increase during ovule development in *V. vinifera* L. cv. Youngle. However, the same gene in V. vinifera L. cv. Pinot noir was initially upregulated, then later downregulated. Following the instructions of the DNA gel extraction kit, the purified PCR products of the 25 day full-bloom stage ovules of *V. vinifera* L. cv. Youngle were recovered and identified. The other products were similarly identified. A total of 24 ESTs were screened, with lengths ranging from 147 bp to 1785 bp. The gene sequences were submitted to GenBank and assigned accession numbers JK818295–JK818319.

3.2. Sequence analysis of candidate genes associated with the development of stenospermocarpic seedless grapes

By using the NCBI sequences to identify homologous sequences, several differentially expressed genes were detected (Table 3). The homologous genes were divided into four categories, including YG1982, YG6623, YG1451, and YG14511 and were absent in the grape and other species. The second category included YG1881, YG1461, YG1731, YG1821, and YG1452, which were all genomic sequences, and no homologous sequences were detected. These

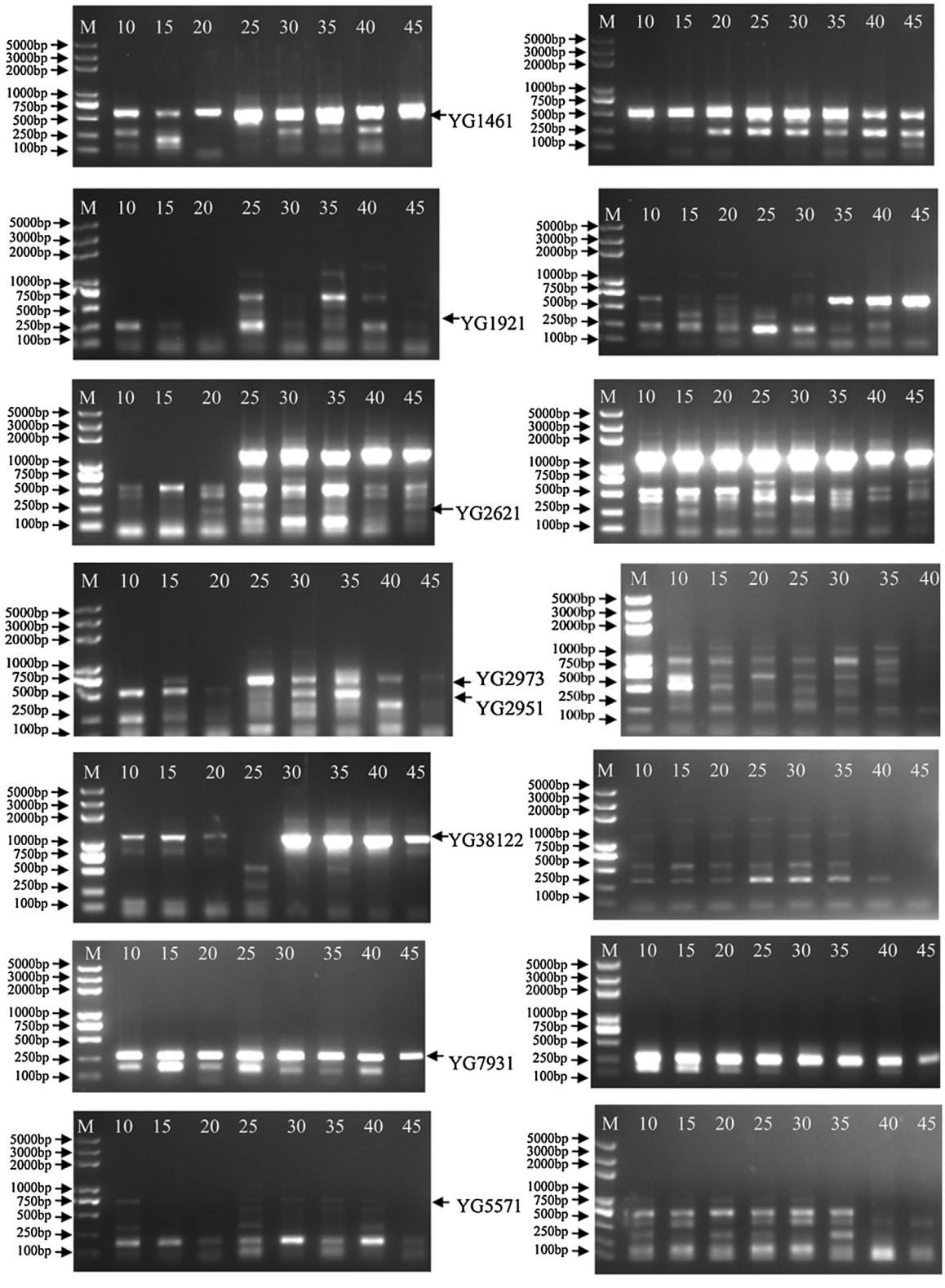

Fig. 1. Different PCR products obtained by SRAP-cDNA. The eight differentially expressed genes were screened in *V. vinifera* L. cv. Youngle (left) and *V. Vinifera* L. cv. Pinot noir (right). The eight candidate genes was examined in ovule development at the following time points: 10 d, 15 d, 20 d, 25 d, 30 d, 35 d, 40 d, and 45 d. YG1461, YG1921, YG2621, YG38122, YG5571, and YG7931 were amplified using primers Me1-Em4, Me1-Em9, Me2-Em6, Me3-Em8, Me5-Em5, and Me7-Em9, respectively. YG2973and YG2951 were amplified using the primer pair, Me2-Em9. M indicates the 2000 bp DNA maker.

discrepancies may be due to the fact that genome annotation was not complete or their patterns of expression might have changed and thus required further verification. The third category included the YG2621, YG5571, and YG7931 predicted sequences in the grape genome. The fourth category included YG2522, which showed high homology with those of the other species, but very low homology with those of grape. This finding might also be due to the incomplete status of genome annotation.

Table 3
Sequence analysis of ovule abortion-related genes in seedless grape.

Sample No	Length	Putative Identification (Organism)	Identity	E-value	Accession No
YG1881	645	genome shotgun sequence [*Vitis vinifera*]	622/626 (99%)	0.0	JK818295
YG1921	239	protein notum homolog-like [*Vitis vinifera*]	216/218 (99%)	2e-105	JK818297
YG1982	147	None			JK818298
YG1736	250	probable methionyl-tRNA synthetase-like [*Vitis vinifera*]	238/241 (99%)	2e-116	JK818299
YG1461	587	genome shotgun sequence [*Vitis vinifera*]	570/577 (99%)	0.0	JK818300
YG6841	430	putative disease resistance protein RGA4-like [*Vitis vinifera*]	412/416 (99%)	0.0	JK818301
YG1463	590	genome shotgun sequence [*Vitis vinifera*]	569/578 (98%)	0.0	JK818302
YG1731	244	genome shotgun sequence [*Vitis vinifera*]	226/232 (97%)	6e-106	JK818303
YG1737	299	genome shotgun sequence [*Vitis vinifera*]	284/286 (99%)	1e-143	JK818304
YG1763	805	retrotransposon V16 [*Vitis vinifera*]	770/785 (98%)	0.0	JK818305
YG2621	221	hydroxyacylglutathione hydrolase cytoplasmic-like [*Vitis vinifera*]	201/203 (99%)	1e-97	JK818306
YG2522	247	RNA polymerase beta' subunit protein (rpoC1) gene [*Berberidopsis corallina*]	195/200 (98%)	6e-91	JK818307
YG2951	544	cyclin-D3-1-like [*Vitis vinifera*]	502/514 (98%)	0.0	JK818308
YG2973	705	peroxidase 12-like [*Vitis vinifera*]	660/667 (99%)	0.0	JK818309
YG5571	753	sorcin-like [*Vitis vinifera*]	704/717 (98%)	0.0	JK818310
YG68115	1256	xanthoxin dehydrogenase-like [*Vitis vinifera*]	1205/1210 (99%)	0.0	JK818311
YG68185	1785	probable celluLose synthase A catalytic subunit 5 [UDP-forming]-like [*Vitis vinifera*]	1727/1738 (99%)	0.0	JK818312
YG6623	261	None			JK818313
YG7931	311	probable WRKY transcription factor 20-like [*Vitis vinifera*]	269/269 (100%)	2e-137	JK818314
YG1821	191	genome shotgun sequence [*Vitis vinifera*]	139/139 (100%)	2e-65	JK818315
YG1451	218	None			JK818316
YG1452	283	genome shotgun sequence [*Vitis vinifera*]	243/268 (91%)	3e-89	JK818317
YG14511	399	None			
YG38122	1127	uncharacterized LOC100266857 [*Vitis vinifera*]	1114/1124 (99%)	0.0	JK818318

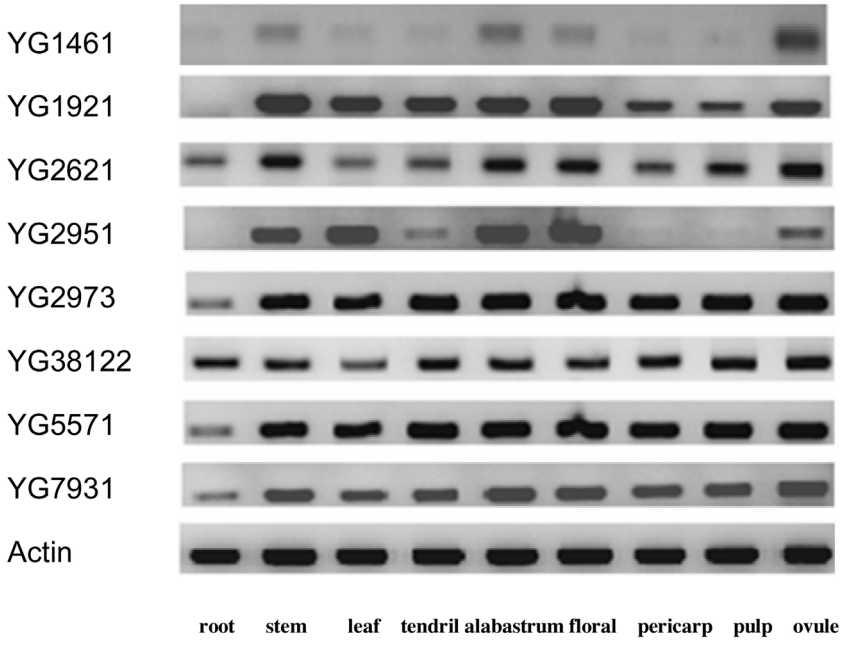

Fig. 2. Expression profiling of 8 candidate genes, YG1461, YG1921, YG2621, YG2951, YG2973, YG38122, YG5571 YG7931, were detected in the root, stem, leaf, tendril, alabastrum, floral, pericarp, pulp, and ovule in *V. vinifera* L. cv. Youngle. YG1921 and YG2951were expressed in specific tissues. YG1461 was expressed low throughout the period besides ovule. The other genes were expressed in all tissues. Expression of the reference gene, actin, was used to normalize the expression values of all candidate genes.

3.3. Expression of the candidate genes in different tissues

After sequence analysis, eight candidate genes were filtered from 24 ESTs. We detected the tissue-specific expression of the candidate genes by PCR to determine whether these were specially expressed during ovule development in *V. vinifera* L. cv. Youngle. The results showed that YG1921 was expressed in all tissues except the root. YG2951 was expressed in all tissues in addition to the root, pericarp, and pulp. YG1461 was expressed low throughout the period besides ovule. The other genes were expressed in all tissues, although the levels of expression varied among different tissues (Fig. 2).

3.4. qRT-PCR analysis of transcription levels of ovules of stenospermocarpic seedless grapes

The 8 genes from the 24 ESTs were further analyzed by qRT-PCR to validate whether these genes were related to ovule development in stenospermocarpic seedless grapes. The actin gene exhibited the best results (Fig. 3). Furthermore, the relative expression patterns of YG1461, YG2621, and YG2973 were similar, whereas that of *V. vinifera* L. cv. Youngle varied. No significant difference in expression level was observed between *V. vinifera* L. cv. Youngle and *V. vinifera* L. cv. Pinot noir, indicating that these genes were not related to ovule development. YG1921and YG2951 showed similar patterns

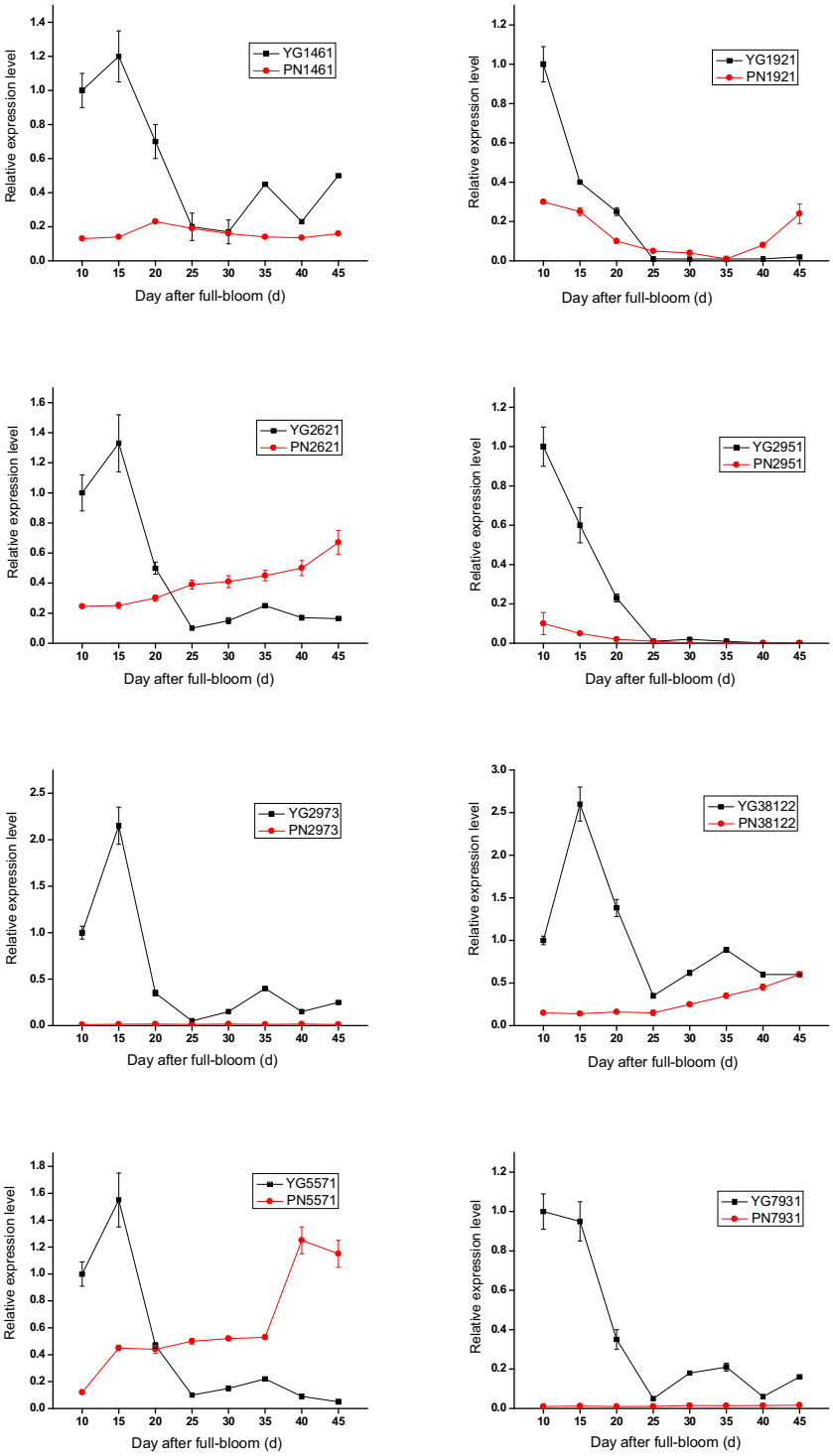

Fig. 3. Expression patterns of the candidate genes at one day after full-bloom in *V. vinifera* L. cv. Youngle and *V. vinifera* L.cv. Pinot noir. The relative expression of the YG1461, YG1921, YG2621, YG2951, YG2973, YG38122, YG5571, and YG7931 were measured by qRT-PCR. The expression pattern of YG38122, YG5571, and YG7931 was different in *V. vinifera* L. cv. Youngle and *V. vinifera* L.cv. Pinot noir. The Y-axes are scales of relative expression levels (error bars indicate ±SD).

of expression in the two grape species, except that these were of different levels, indicating that these genes were not related to ovule development. The expression pattern of YG38122, YG5571, and YG7931 in *V. vinifera* L. cv. Youngle was of the converse "S" type, whereas an increasing trend was observed in the *V. vinifera* L. cv. Pinot noir. These results indicated that YG38122, YG5571, and YG7931 were related to ovule development.

4. Discussion

The central dogma of molecular biology states that mRNA serves as the bridge between DNA and protein. Gene expression is primarily controlled at the level of transcription. Therefore, research studies involving transcription levels can reveal the relationship between a gene and a specific biological process. In the present study, SRAP was employed as a molecular marker tech-

nique to determine the expression of a gene at the transcriptional level. Liu et al. (2013) applied cDNA-SRAP to isolate differentially expressed genes between the two species during fiber development as well as to detect differences in the mechanism underlying fiber development between *G. hirsutum* and *G. barbadense*. Huo et al. (2012) investigated differential gene expression in the flower buds of male-sterile and male-fertile pools by using the cDNA-SRAP method and discovered a fragment that was specifically expressed in the male-fertile pool Therefore, cDNA-SRAP can be used to study genes that are associated with stenospermocarpic seedless. In the present study, eight candidate genes were selected for qRT-PCR including that of the ovule upon reaching the full-bloom stage and expression profiling in different tissues. The present study determined that the YG38122, YG5571, and YG7931 genes were related to the development of the stenospermocarpic seedless grape. This also showed that cDNA-SRAP can be used for screening genes that are involved in ovule development.

Among the eight genes, three candidates were selected based on the results of qRT-PCR analysis. The putative gene, YG38122, encodes an unknown protein that has not been characterized to date. It is expressed in all tissues, although its expression pattern varied between *V. vinifera* L. cv. Youngle and cv. Pinot noir. Based on these findings, we deduced that YG38122 is related to ovule development.

The second candidate gene, YG5571, has two EF-hand motifs (calcium-binding motif) and encodes the protein, sorcin, which has been recently identified in a wide range of human cells, including cardiac cells, vascular smooth cells, and adrenal medulla (Anthony et al., 2007; Beyer-Sehlmeyer et al., 1999). In addition, sorcin has also been identified in various tumor cells such as leukemia, gastric cancer, breast cancer, and ovarian cancer (Chuthapisith et al., 2009; Deng et al., 2010; Parekh et al., 2002; Qi et al., 2006). However, research studies on sorcin in plants are limited. Previous studies have indicated that proteins harboring EF-hand motifs play a critical role in animal embryonic development (Gersdorff et al., 2006; Webb and Miller, 2003). In addition, some genes with a Ca^{2+}-binding EF-hand are related to lipid bodies in developing plant embryos and mature seeds such as caleosins (CLO) (Næsted et al., 2000). The VvCBP with two EF-hand motifs has been determined to play an important role in the embryonic development of the seedless grapevine. The silencing of its homologous gene results in a significant decrease in the number of seeds in tomato (Wang et al., 2011). Moreover, earlier studies have indicated that calcium-dependent protein kinase (CDPK) and Ca^{2+} might play regulatory roles in oil accumulation/oil body biogenesis during seed maturation (Anil et al., 2003). The present study determined that YG5571 was expressed in all tissues, although its patterns were highly variable. qRT-PCR revealed that a high amount of YG5571 mRNA accumulated in *V. vinifera* L. cv. Pinot noir, whereas this was downregulated in *V. vinifera* L. cv. Youngle. These findings suggest that YG5571 might be related to ovule development.

The third candidate gene, YG7931, encodes a WRKY transcription factor. The WRKY family of proteins is a class of plant-specific transcription factors that are involved in plant stress response, growth, development, and senescence. Previous studies have shown that specific WRKY transcription factors are involved in seed development. Mutants of the novel gene, *TTG2*, which encodes a WRKY transcription factor, show disruptions in trichome development, as well as in tannin and mucilage production in the seed coat (Johnson et al., 2002). ScWRKY1 is strongly and transiently expressed in fertilized ovules bearing late torpedo-staged embryos in developing seeds, which indicates its specific role in embryogenesis (Lagace and Matton, 2004). AtWRKY44 is involved in seed coat development and is upregulated in the endothelial cells of normal seeds, but downregulated in mutants (Johnson et al., 2002). Subsequent research has shown that the programming of the mother cell regulates the lethality of the interploid hybrid offspring (Dilkes et al., 2008). AtWRKY10 is expressed in pollen, in the developing endosperm, as well as in the globular embryo, but not during the late heart stage of embryo development. Mutant alleles of this gene produce a small seed phenotype that is associated with reduced growth and early cellularization of the endosperm (Luo et al., 2005). In the present study, YG7931 was expressed in all tissues, although a different pattern of expression was observed in the ovule of *V. vinifera* L. cv. Youngle, which was downregulated, and *V. vinifera* L.cv. Pinot noir, which was upregulated. These findings indicate that YG7931 is related to ovule development.

5. Conclusion

cDNA-SRAP is an effective method for screening candidate genes involved in ovule development. A total of 24 ESTs were detected and further classified into four categories. The first category included YG1982, YG6623, YG1451, and YG14511; the second category consisted of YG1881, YG1461, YG1731, YG1821, and YG1452; the third category comprised YG2621, YG5571, and YG7931; and the fourth category included YG2522. Sequence analysis allowed us to filter eight candidate genes from 24 ESTs, which included YG1461, YG1921, YG2621, YG38122, YG5571, YG7931, YG2973, and YG2953. Through specific expression in different tissues and qRT-PCR analysis of different ovule developmental stages, we identified three genes, YG38122, YG5571, and YG7931. The expression pattern of these genes was of the converse "S" type in *V. vinifera* L. cv. Youngle, whereas that *V. vinifera* L. cv. Pinot noir showed an increasing pattern, indicating that YG38122, YG5571, and YG7931 are involved in ovule development.

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References

Anil, V.S., Harmon, A.C., Rao, K.S., 2003. Temporal association of Ca^{2+}-dependent protein kinase with oil bodies during seed development in *Santalum album* L.: its biochemical characterization and significance. Plant Cell Physiol. 44, 367–376.

Anthony, D.F., Beattie, J., Paul, A., Currie, S., 2007. Interactionof calcium/calmodulin-dependent protein kinase IIδC with sorcin indirectly modulates ryanodine receptor function in cardiac myocytes. J. Mol. Cell Cardiol. 43, 492–503.

Beyer-Sehlmeyer, G., Hiddemann, W., WÖrmann, B., Bertram, J., 1999. Suppressive subtractive hybridisation reveals differential expression of serglycin, sorcin, bone marrow proteoglycan and prostate-tumour-inducing gene I (PTI-1) in drug-resistant and sensitive tumour cell lines of haematopoetic origin. Eur. J. Cancer 35, 1735–1742.

Cabezas, J.A., Cervera, M.T., Ruiz-García, L., Carreño, J., Martínez-zapater, J.M., 2006. A genetic analysis of seed and berry weight in grapevine. Genome 12, 1572–1585.

Chuthapisith, S., Bean, B.E., Cowley, G., Eremin, J.M., Samphao, S., Layfield, R., Kerr, I.D., Wiseman, J., El-Sheemy, M., Sreenivasan, T., Eremin, O., 2009. Annexins in human breast cancer:possible predictors of pathological response to neoadjuvant chemotherapy. Eur. J. Cancer 45, 1274–1281.

Costenaro-da-Silva, D., Passaia, G., Henriques, J.A., Margis, R., Pasquali, G., Revers, L.F., 2010. Identification and expression analysis of genes associated with the early berry development in the seedless grapevine (*Vitis vinifera* L.) cultivar sultanine. Plant Sci. 179, 510–519.

Deng, X.H., Zhang, S.N., Hou, X.L., 2007. Differential expression analysis of bud of pol CMS and its maintainter line of *Brassica rapa* ssp. chinensis through SRAP. Acta Hortic. Sin. 34, 655–658.

Deng, L., Su, T., Leng, A., Zhang, X., Xu, M., Yan, L., Gu, H., Zhang, G., 2010. Upregulation of soluble resistance-related calcium binding protein (sorcin) in gastric cancer. Med. Oncol. 27, 1102–1108.

Dilkes, B.P., Spielman, M., Weizbauer, R., Watson, B., Burkart-Waco, D., Scott, R.J., Comai, L., 2008. The maternally expressed WRKY transcription factor TTG2 controls lethality in interploidy crosses of Arabidopsis. PLoS Biol. 6, 2707–2720.

Doligez, A., Bouquet, A., Danglot, Y., Lahogue, F., Riaz, S., Meredith, C.P., Edwards, K.J., This, P., 2002. Genetic mapping of grapevine (*Vitis vivifera*. L.) applied to

the detection of QTLs for seedless and berry weight. Theor. Appl. Genet. 105, 780–795.
Dudley, J.W., 1993. Molecular markers in plant improvement manipulation of genes affecting quantitiative. Crop Sci. 33, 660–668.
Fischer, B.M., Salakhutdinov, I., Akkurt, M., Eibach, R., Edwards, K.J., Töpfer, R., Zyprian, E.M., 2004. Quantitative trait locus analysis of fungal disease resistance factors on a molecular map of grapevine. Theor. Appl. Genet. 108, 501–515.
Gersdorff, N., Mller, M., Schall, A., Miosge, N., 2006. Secreted modular calcium binding protein-1 localization during mouse embryogenesis. Histochem. Cell Biol. 126, 705–712.
Hanania, U., Velcheva, M., Or, E., Flaishman, M., Sahar, N., Perl, A., 2007. Silencing of chaperonin 21, that was differentially expressed in inflorescence of seedless and seeded grapes, promoted seed abortion in tobacco and tomato fruits. Transgenic Res. 16, 515–525.
Huo, Y.M., Miao, J., Liu, B.I., Yang, Y.Y., Zhang, Y.H., TAHARA, Y., Meng, Q.W., KITANO, H., Wu, X., 2012. The expression of pectin methylesterase in onion flower buds is associated with the dominant male-fertility restoration allele. Plant Breed. 131, 211–216.
Ingrosso, I., Bonsegna, S., Domenico, S.D., Laddomada, B., Blando, F., Santino, A., Giovinazzo, G., 2011. Over-expression of a grape stilbene synthase gene in tomato induces parthenocarpy and causes abnormal pollen development. Plant Physiol. Biochem. 49, 1092–1099.
Johnson, C.S., Kolevski, B., Smyth, D.R., 2002. TRANSPARENT TESTA GLABRA2, a trichome and seed coat development gene of Arabidopsis, encodes a WRKY transcription factor. Plant Cell 14, 1359–1375.
Lagace, M., Matton, D.P., 2004. Characterization of a WRKY transcription factor expressed in late torpedo-stage embryos of Solanum chacoense. Planta 219, 185–189.
Ledbetter, C.A., Ramming, D.W., 2011. Seedlessness in grapes. Hortic. Res. 11, 159–184.
Ledbettter, C.A., Burgos, L., 1994. Inheritance of stenospermocarpic seedlessness in *Vitis vinifera* L. J. Hered. 2, 157–160.
Li, G., Quiros, C.F., 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction:its application to mapping and gene tagging in Brassica. Theor. Appl. Genet. 103, 455–461.
Liu, S.M., Clingeleffer, S.R.S., 2008. Effect of culture medium, genotype, and year of cross on embryo development and recovery from in vitro cultured ovules in breeding stenospermocarpic seedless grape varieties. Aust. J. Agric. Res. 59, 175–182.
Liu, C.X., Yuan, D.J., Zhang, X.L., Lin, Z.X., 2013. Isolation: characterization and mapping of genes differentially expressed during fibre development between *Gossypium hirsutum* and *G. barbadense* by cDNA-SRAP. J. Genet. 92, 175–181.
Lu, Y.Q., Wu, W.R., 2006. Identification of salt-responsive genes in English cordgrass (*Spartina anglica*) roots using SRAP technique. J. Zhejiang Univ. 32, 511–514.
Luo, M., Dennis, E.S., Berger, F., Peacock, W.J., Chaudhury, A., 2005. MINISEED3 (MINI3), a WRKY family gene, and HAIKU2 (IKU2) a leucine-rich repeat (LRR) KINASE gene, are regulators of seed size in Arabidopsis. Proc. Natl. Acad. Sci. U. S. A. 102, 17531–17536.
Mejia, N., Soto, B., Guerrero, M., Casanueva, X., Houel, C., Miccono, M.L., Ramos, R., Cunff, L.L., Boursiquot, J.M., Hinrichsen, P., Adam-Blondon, A.F., 2011. Molecular, genetic and transcriptional evidence for a role of VvAGL11 in stenospermocarpic seedlessness in grapevine. BMC Plant Biol. 11, 29–33.
Næsted, H., Frandsen, G.I., Jauh, G.Y., Hernandez-Pinzon, I., Nielsen, H.B., Murphy, D.J., Rogers, J.C., Mundy, J., 2000. Caleosins: ca $^{2+}$-binding proteins associated with lipid bodies. Plant Mol. Biol. 44, 463–476.
Notsuka, K., Tsuru, T., Shiraishi, M., 2001. Seedless-seedless grape hybridization via in-ovulo embryo culture. Engei Gakkai Zasshi. 70, 7–15.
Parekh, H.K., Deng, H.B., Choudhary, K., Houser, S.R., Simpkins, H., 2002. Overexpression of sorcin, a calcium-binding protein,induces a low level of paclitaxel resistance in humanovarian and breast cancer cells. Biochem. Pharmacol. 63, 1149–1158.
Qi, J., Liu, N., Zhou, Y., Tan, Y., Cheng, Y., Yang, C., Zhu, Z., Xiong, D., 2006. Overexpression of sorcin in multi drugresistant human leukemia cells and its role in regulating cell apoptosis. Biochem. Biophys. Res. Commun. 349, 303–309.
Roytchev, V., 1998. Inheritance of grape seedlessness in seeded and seedless hybrid cominations of grape cultivars with complex genealogy. Am. J. Enol. Vitic. 49, 302–305.
Sarikhani, H., Wakana, A., Peterlunger, E., Gaspero, G.D., Cipriani, G., 2009. Effect of ploidy on parthenocarpy in grape cultivars. Acta Hortic. 827, 433–438.
Stout, A.B., 1936. Seedlessness in Grapes. New York State Agricultural Experiment Station, New York.
Wang, S., Yu, Y., Zhang, C., Xu, W., Wang, Y., 2011. Molecular cloning and characterization of a novel gene encoding an EF-hand calcium-binding protein related to fruit seedlessness of grapevine. Sci. Hortic. 130, 708–714.
Webb, S.E., Miller, A.L., 2003. Calcium signalling during embryonic development. Nat. Rev. Mol. Cell. Biol. 4, 539–551.
Zhang, J.J., Wang, Y.J., Wang, X.P., Yang, K.Q., Yang, J.X., 2003. An improved method for rapidly extracting total RNA from vitis. J. Fruit Sci. 20, 178–181.