阳光玫瑰葡萄生长期花芽分化形态进程及相关生理分子水平变化研究

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摘 要：【目的】探讨阳光玫瑰葡萄生长期花芽分化进程及相关生理分子水平变化，为葡萄生产调控及花芽分化深入研究提供理论参考。【方法】以4年生阳光玫瑰葡萄为试材，通过徒手剥离冬芽鳞片在体式解剖镜下观察冬芽形态结构变化，测定花芽分化过程中第5节位叶片内碳水化合物、矿质元素含量及冬芽内9个成花关键基因的表达。【结果】南宁地区阳光玫瑰葡萄在新梢6片展叶期时开始花芽形态分化，在末花期进入花序原基分化期。叶片可溶性总糖、淀粉含量在花序原基分化期后显著升高。叶片P、K、Ca和Mg元素含量在花芽形态分化起始时下降，在花序原基分化后60 d时含量显著降低。冬芽中*VvFT*和*VvSOC1*基因在花芽分化起始时表达水平较高；*VvLFY*、*VvAP1*、*VvFUL*、*VvAP2*、*VvAP3*和*VvAG*基因均在花序原基分化期及花序原基分化期后80~100 d出现表达波峰，*VvFLC*基因在花序原基分化后60~100 d的表达水平较高。【结论】南宁地区阳光玫瑰葡萄花芽分化进程开始较早。生产上在果实膨大期和软化期前后应适当补充磷、钾、钙、镁肥，以促进果实发育、花序原基及其各级穗轴分化。*VvFT*和*VvSOC1*基因参与诱导始原基及花序原基分化，*VvLFY*、*VvAP1*、*VvFUL*、*VvAP2*、*VvAP3*和*VvAG*基因可能均参与促进花序原基及其各级穗轴分化。

关键词：阳光玫瑰葡萄；花芽分化；矿质元素；碳水化合物；基因表达

中图分类号：S663.1　　　文献标志码：A　　　文章编号：1009-9980(2023)01-0074-14

A study on the morphological process and physiological and molecular changes of flower bud differentiation in Shine Muscat grape during fruit growing season

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Abstract:【Objective】In order to provide a theoretical reference for the regulation and the further study of the flower bud differentiation mechanism of grape, the flower bud differentiation process of Shine Muscat grape and the physiological changes and relative gene expression in fruit growth stage were studied.【Methods】4-year-old Shine Muscat grape trees were used as the materials. During the growing season, twenty winter buds at the fifth node of the new shoots were randomly collected every three days from the five leaves separating stage with three repeats. The dissection of the lamella of winter buds was performed by hand under stereomicroscope to observe the morphological changes of the

flower bud differentiation process. The differentiation stages of each bud were counted and recorded. The flower bud differentiation of Shine Muscat was divided into six stages, namely leaf bud stage, flat top stage, dichotomy stage, anlagen differentiation stage, inflorescence primordium differentiation stage and inflorescence branching axis differentiation stage. Each differentiation stage was determined according to the flower bud differentiation stage of over 75% of the sampled winter buds. After the inflorescence primordium differentiation stage, the samplings were carried out every 20 days until the fruit harvest. Phenological periods were recorded for each flower bud differentiation stages and sampling days after the inflorescence primordium differentiation stage. Meanwhile, twenty winter buds were collected to determine the expression of nine key flowering genes *VvFT*, *VvSOC1*, *VvLFY*, *VvAP1*, *VvFUL*, *VvAP2*, *VvAP3*, *VvAG* and *VvFLC*, five leaves at the fifth node of the branch were sampled for the determination of total soluble sugar, starch, mineral elements N, P, K, Ca and Mg contents with three repeats. 【Results】The results showed that the flower bud differentiation of Shine Muscat winter buds started at the sixth leaf separating stage, and entered the anlagen differentiation stage at the full-blooming stage, then entered inflorescence primordium differentiation stage at the end of flowering stage. The content of N in the leaves maintained at 2.5−3.0 g·kg^{-1} from the leaf bud stage to the inflorescence primordium differentiation stage, and then reached the lowest value at the stage of 20 days after inflorescence primordium differentiation. The changes of P, K, Ca and Mg content in the leaves were the same, these elements decreased at the beginning of morphological differentiation, and then increased twice. The first peak was found at the inflorescence primordium differentiation stage for P and Mg and at the stage of 40 days after inflorescence primordium differentiation for K, and the second peak was found at the stage of 100 days after inflorescence primordium differentiation for P, K and Mg. The content of Ca in the leaves showed an overall upward trend, with a low content at the leaf bud stage and flat top stage, a significant rise at dichotomy stage, and two peaks appeared at the stage of 40 and 100 days after the inflorescence primordium differentiation. Overall, the contents of P, K, Ca and Mg in the leaves decreased significantly at the stage of 60 days after the inflorescence primordium differentiation. The flower induction genes *VvFT* and *VvSOC1* were highly expressed during the flower buds differentiation induction stage. The expression of the *VvFT* reached the first peak at the flat top stage and the second peak at the stage of 20 days after the inflorescence primordium differentiation. The expression of the *VvSOC1* was significantly up-regulated at the flat-top stage and reached the peak at the dichotomy stage, but kept a low level after the stage of 40 days after the inflorescence primordium differentiation. The expression of the *VvLFY*, *VvAP1* and *VvFUL* were all at a low level during the early stages of flower bud differentiation. The expression of the *VvLFY* reached a peak at the stage of 80 days after the inflorescence primordium differentiation. The expression of the *VvAP1* increased from the stage of inflorescence primordium differentiation, and reached the highest level at the stage of 20 and 100 days after the inflorescence primordium differentiation. The expression level of the *VvFUL* increased at the stage of the inflorescence primordium differentiation, and reached the highest level at the stage of 80 days after the inflorescence primordium differentiation. The expression of the *VvAP2* reached the highest level at the inflorescence primordium differentiation stage, and showed a significant upward trend from the stage of 40 to 100 days after the inflorescence primordium differentiation. During the flower bud differentiation, the expression trends of the *VvAP3* and *VvAG* were consistent, the expressions of the *VvAP3* and *VvAG* were down-regulated at the beginning of the flower bud differentiation, and reached highest level at the stage of 80 days after the inflorescence primordium differentiation. The expression level of the *VvFLC* was significantly lower from the inflorescence primordium differentiation stage to the stage of 40 days

after the inflorescence primordium differentiation, but exhibited a relatively high level during the stages of 60 to 100 days after the inflorescence primordium differentiation. 【Conclusion】The flower bud differentiation process of Shine Muscat grape was earlier in Nanning. More organic nutrition and main mineral elements were demanded during the inflorescence branching axis differentiation stage. In production, during the stage of before and after the enlargement and softening of Shine Muscat grapes, appropriate supplements of phosphorus, potassium, calcium and magnesium fertilizers are important to promote fruit development and normal differentiation of inflorescence branching axis. The *VvFT* and *VvSOC1* were involved in the induction of primordium and inflorescence primordium differentiation. The *VvLFY*, *VvAP1*, *VvFUL*, *VvAP2*, *VvAP3* and *VvAG* may be involved in the inflorescence primordium differentiation and further differentiation of development of branching axis. The high expression of the *VvFLC* may inhibit the expression of flower induction genes, during inflorescence branching axis differentiation stage.

Key words: Shine Muscat grape; Flower bud differentiation; Mineral element; Carbohydrate; Gene expression

葡萄花芽分化是生长发育过程中十分重要的阶段,花芽分化的数量及质量影响葡萄产量及经济效益[1]。葡萄的成花过程包括成花诱导(flower induction)、花的发端(flower initiation)即始原基分化、花序分化和花器官分化(inflorescence and flower organization)、花的成熟(flower maturation)和开花(anthesis)等过程[2]。葡萄花芽分化可大致分为两个过程,即生理分化和形态分化,成花诱导即为通常所称的生理分化,始原基分化、花序分化和花器官分化即通常所称的形态分化[2-3]。葡萄花芽形态分化跨越了两个生长季,花序分化一直持续到冬季休眠,第二季萌芽前转入花器官分化阶段[4]。

葡萄花芽分化是一个较为复杂的形态建成过程,受诸多因素影响,包括环境因素如光照、温度、水分和内部因素如营养水平、内源激素水平、基因调控等[4]。光照通过影响叶片光合作用和光合产物积累等间接影响葡萄花芽分化,光照与温度协同促进花芽分化[5]。葡萄花序原基诱导和分化通常要求温度在20 ℃以上,温度低于20 ℃始原基则发育形成卷须原基[6-8]。水分通过影响细胞分裂和膨大及树体光合作用、营养水平、内源激素水平及芽内微域环境直接或间接影响花芽分化[4]。葡萄中碳水化合物与花芽分化呈正相关,充足的糖和淀粉可促进葡萄花芽分化[9]。葡萄成花诱导及花序分化需要N和P元素供应[10-11]。高水平的P、K、Ca、Mg和低水平的N元素有利于葡萄花芽分化[12-13]。葡萄花芽分化还与内源激素密切相关,赤霉素和细胞分裂素是调控葡萄成

花的主要激素[5]。赤霉素在葡萄花芽分化过程中抑制葡萄花序原基分化,细胞分裂素能够促进葡萄成花[14-15]。

葡萄花芽分化过程中,相关基因的表达与互作调控其成花。目前,葡萄中报道了很多与模式植物拟南芥同源的花芽分化相关基因,并且大多数基因功能相似[16]。*FLOWERING LOCUS T*(*FT*)、*SUPPRESSOR OF CONSTANS 1*(*SOC1*)和*LEAFY*(*LFY*)是重要的开花整合因子,参与调控成花时间[17-19]。在葡萄中,*VvFT*基因在芽、叶片、花序、卷须及幼果等器官中均有表达,参与了花序与花器官分化及发育[20-21]。*VvSOC1*基因在葡萄花序原基和苞片中的表达处于较高水平[22]。*LEAFY*(*LFY*)基因既是花分生组织特征基因,也是整合环境信号和内源信号的重要开花控制因子[23]。*VvLEAFY*基因在葡萄花序原基发育过程的表达水平较高,参与调控花序和花分生组织的发育[24]。*APETALA1*(*AP1*)及其旁系同源基因*FRUITFULL*(*FUL*)既是花分生组织特征基因,也作为花器官特征基因促进萼片和花瓣的发育[16]。葡萄中*VvAP1*和*VvFUL*基因在花序和花分生组织中表达[25]。*APETALA2*(*AP2*)、*APETALA3*(*AP3*)和*AGAMOUS*(*AG*)为花器官特征基因,调控花瓣、雄蕊、心皮及胚珠的发育[26-27]。*VvAP2*基因在葡萄叶、茎、花序、花等器官中均有表达,其在花序和花中的表达量较高[28]。*VvAG*基因在葡萄花序、果实、花瓣和雄蕊中表达[29]。*FLOWERING LOCUS C*(*FLC*)为成花抑制因子,*FLC*基因通过抑制成花基因*FT*和

SOC1 的表达进而抑制成花[30]。刘丹等[31]基于EST数据库研究发现葡萄中 *VvFLC* 抑制 *VvFT*、*VvSOC1* 和 *VvLFY* 基因的表达。

迄今，关于葡萄成花的相关生理及分子研究大部分集中于花芽分化关键时期，然而葡萄当季新梢上冬芽的花芽分化过程与果实的发育期重叠，在果实的生长发育期，尤其是花序原基分化后到果实采收期间成花相关的生理分子变化未见详细报道。阳光玫瑰葡萄是近年中国葡萄产业新兴推广的品种，风味品质独特，具有很好的发展前景，然而其花芽分化相关研究还鲜有报道。笔者在本研究中以阳光玫瑰葡萄为试材，研究葡萄生长期新梢上冬芽花芽分化的形态结构及可溶性糖、淀粉、矿质元素含量和成花关键基因表达的变化，揭示阳光玫瑰葡萄成花进程及花芽分化相关机制，以期在温光资源丰富的热区为葡萄的生产调控及其花芽分化的深入研究提供理论依据。

1　材料和方法

1.1　试验材料及试验设计

试验于2019年3月至2019年9月在广西南宁广西真诚农业有限公司武鸣东盟开发区示范基地进行。以4年生阳光玫瑰葡萄为试材，果园管理采用当地常规一年两收栽培管理方式，树体生长良好。

在阳光玫瑰葡萄生长期，从新梢5片展叶期开始每隔3 d随机采新梢第5节位冬芽20个，3次重复，采样当天即在体式解剖镜下徒手剥离冬芽鳞片观察花芽形态结构，统计并记录各芽所处的分化阶段，花芽分化阶段划分参照王海波等[32]和林玲[33]的研究，将阳光玫瑰冬芽花芽分化时期划分为叶芽期、平顶期、二分体期、始原基分化期、花序原基分化期、花序各级穗轴分化期。当超过75%的冬芽处于上述某一分化阶段时确定该次采样期进入该分化阶段，在花序原基分化期后，花序各级穗轴分化期每隔20 d采样1次继续观察冬芽花芽形态结构变化，至夏果果实采收时结束采样。

在观察到叶芽期、平顶期、二分体期、始原基分化期、花序原基分化期的采样当天及花序各级穗轴分化期的每隔20 d 1次的采样当天，记录物候期，并采集碳水化合物、矿质元素含量测定和成花关键基因表达分析的样品。碳水化合物和矿质元素含量测定随机采集枝条第5节位叶片5枚，置于冰盒临时保

存，回实验室后放入烘箱中108 ℃杀青20 min，后65 ℃烘干，将样品研磨成粉末后装袋保存，重复3次；成花关键基因表达分析随机采集新梢第5节位冬芽20个，芽切下后立即冻入液氮临时保存，后置于-80 ℃保存，3次重复。

1.2　测定方法

1.2.1　花芽分化过程芽形态结构观察　在体式解剖镜下剥去鳞片，观察葡萄花芽分化不同阶段芽的形态结构。

1.2.2　叶片可溶性总糖、淀粉含量测定　可溶性总糖及淀粉的提取及含量测定采用蒽酮乙酸乙酯-硫酸比色法。

1.2.3　叶片矿质元素含量测定　全N含量的提取与测定。用 H_2SO_4-H_2O_2 消煮法提取样品，使用连续流动分析仪进行测定。全P、K、Ca、Mg含量的提取与测定。用 H_2NO_2-H_2O_2 消煮法提取样品，使用电感耦合等离子光谱仪进行测定。

1.2.4　芽内9个成花关键基因表达量测定　葡萄冬芽总RNA的提取采用RNAprep Pure Plant Plus kit（TIANGEN）总RNA提取试剂盒进行，用不含RNA酶的DNA酶去除DNA。用 PrimeScript™ RTase 反转录试剂盒进行反转录，合成第一链cDNA，荧光定量采用 TB Green ®Premix Ex *Taq*™ Ⅱ（TaKaRa）试剂盒进行。以葡萄 *ACTIN* 作为内参基因，引物序列参考 Kobayashi 等[34]，9个成花关键基因 *VvFT*、*VvSOC1*、*VvLFY*、*VvAP1*、*VvFUL*、*VvAP2*、*VvAP3*、*VvAG* 和 *VvFLC* 的引物参考杨光等[35]的报道，具体引物序列见表1。试验中每个样品3次重复，同时设置3个阴性对照，目的基因的表达量采用相对定量法（$2^{-\Delta\Delta CT}$）进行计算。

1.3　数据分析

试验数据运用 Excel 2010 进行整理及制图，运用 SPSS 软件进行显著性分析。

2　结果与分析

2.1　阳光玫瑰葡萄冬芽花芽分化过程形态结构观察及其对应的物候期

参考前人研究结果，根据阳光玫瑰葡萄冬芽分化过程中的形态结构变化对其花芽分化进程进行划分，将第5节位冬芽花芽分化各阶段与葡萄树体所处物候期对应见图1，分为以下几个阶段：叶芽期，生长点还未开始分化，尖端突起；平顶期，开始分化

表 1　引物序列

Table 1　Sequence of primers

基因名称 Gene name	上游引物序列（5'-3'） Forward primer (5'-3')	下游引物序列（5'-3'） Reverse primer (5'-3')
ACTIN	GATTCTGGTGATGGTGTGAGT	GACAATTTCCCGTTCAGCAGT
VvFT	GTGTTAGCCTAAACCAAGCT	ACGATTCATTAAGACCGAAAC
VvSOC1	GTCCATGCTCAGCAACACTG	GCAGGGCAATTTACATCAT
VvLFY	TCACGGTGAGTAGGCTTCTG	GGCGTGACTCTTCTTCATCC
VvAP1	CACATGGAGCAAGGAGGAAT	TCACCCAAGTAGATATCAAATCAG
VvFUL	AGTCCCAATACCCTCATGCCACC	GGGGCATTAAAGACTTGGCTGAC
VvAP2	AGCCCCACCATACCATGTAA	CCTCTTCATATCCCCCAACA
VvAP3	CAGGCAAACAATCATCCAGA	AACCTCCCGCTGTCAAGTTA
VvAG	TGTGGGGATGATCTAGTGCC	GACCTATGCCCATCAACAAAGA
VvFLC	AGTGGAAGGCCAAGTGTTTG	TACAGCTCCTGCTTGATTCG

的标志,生长点扁平;二分体时期,生长点开始分化为两个半球状;始原基分化期,生长点旁始原基形成;花序原基分化期,分化出花穗主轴,花序原基形成;花序各级穗轴分化期(花序原基分化后20~100 d),花序原基进一步分化和发育形成各级穗轴。

　　从阳光玫瑰葡萄各采样期冬芽分化进程对应的夏果物候期(表2)可以看出,阳光玫瑰葡萄在新梢6片展叶期时花芽分化处于平顶期即冬芽开始形态分化;葡萄盛花期时冬芽花芽分化进入始原基分化期,该阶段始原基可分化为卷须原基或花序原基,因此,盛花期是阳光玫瑰葡萄花芽形成的关键时期;在葡萄末花期时冬芽进入花序原基分化期;从花序原基分化期后到夏果采收期观察到的花芽形态变化可以看出,花序原基不断发育并逐渐分化出花序各级穗轴。

2.2　阳光玫瑰葡萄冬芽花芽分化过程中叶片可溶性总糖和淀粉含量的变化

　　如图2所示,在阳光玫瑰葡萄冬芽花芽分化过程中,叶片可溶性总糖和淀粉含量整体呈上升趋势,叶片中可溶性总糖含量在花芽分化平顶期即冬芽开始形态分化时显著升高,而后至始原基分化期其含量(w,后同)无显著变化,均保持在3.5 μg·g⁻¹左右,从花序原基分化期开始至花序原基分化后100 d期间可溶性总糖含量一直呈显著上升趋势。叶片淀粉含量从花芽分化平顶期时开始显著升高,在花序原基分化期后20 d达到最高值(141.38 μg·g⁻¹),此时对应的物候期为幼果期,随后叶片淀粉含量有所下降,但均保持在100 μg·g⁻¹以上。以上结果表明,阳光玫瑰葡萄花芽形态分化起始需要一定的可溶性总糖和淀粉,花芽分化过程对可溶性总糖和淀粉含量的需求整体呈上升趋势,保持较高水平的可溶性总糖和淀粉利于花序原基及其各级穗轴分化。

2.3　阳光玫瑰葡萄冬芽花芽分化过程中叶片矿质元素N、P、K、Ca和Mg含量的变化

　　图3为阳光玫瑰葡萄冬芽花芽分化过程中叶片各矿质元素含量的变化。冬芽叶芽期到花序原基分化期叶片全N含量维持在2.5~3.0 g·kg⁻¹,其在花序原基分化后20 d时最低,这可能与此时果实发育N需求增加有关,花序原基分化后40 d时N含量迅速上升并维持在3.0~4.0 g·kg⁻¹,较高水平的N可能有利于花序原基各级穗轴分化和发育。在冬芽花芽分化过程中叶片P、K和Mg含量变化趋势基本一致,平顶期叶片中P、K和Mg含量较叶芽期显著下降,此后出现两次波峰,P和Mg含量在花序原基分化期达到第一个波峰,K在花序原基分化期后40 d达到第一个波峰,三者均在花序原基分化期后100 d时达到第二个波峰。以上表明葡萄花芽分化起始对P、K和Mg需求较大,花序原基分化期后100 d时叶片P、K和Mg含量较高可能与采收期果实对矿质营养需求减少有关。在花芽分化过程中叶片Ca含量整体呈上升趋势,叶芽期及平顶期Ca含量相对较低,于二分体时期开始显著升高,此后Ca含量分别在花序原基分化后40 d和花序原基分化后100 d出现两个波峰,表明较高水平的Ca可能有利于促进花芽进入始原基分化阶段。叶片中P、K、Ca和Mg含量均在花序原基分化后60 d即果实软化期显著下降,这可能与软化期果实发育也需要消耗较多的P、K、Ca和Mg等营养元素有关。
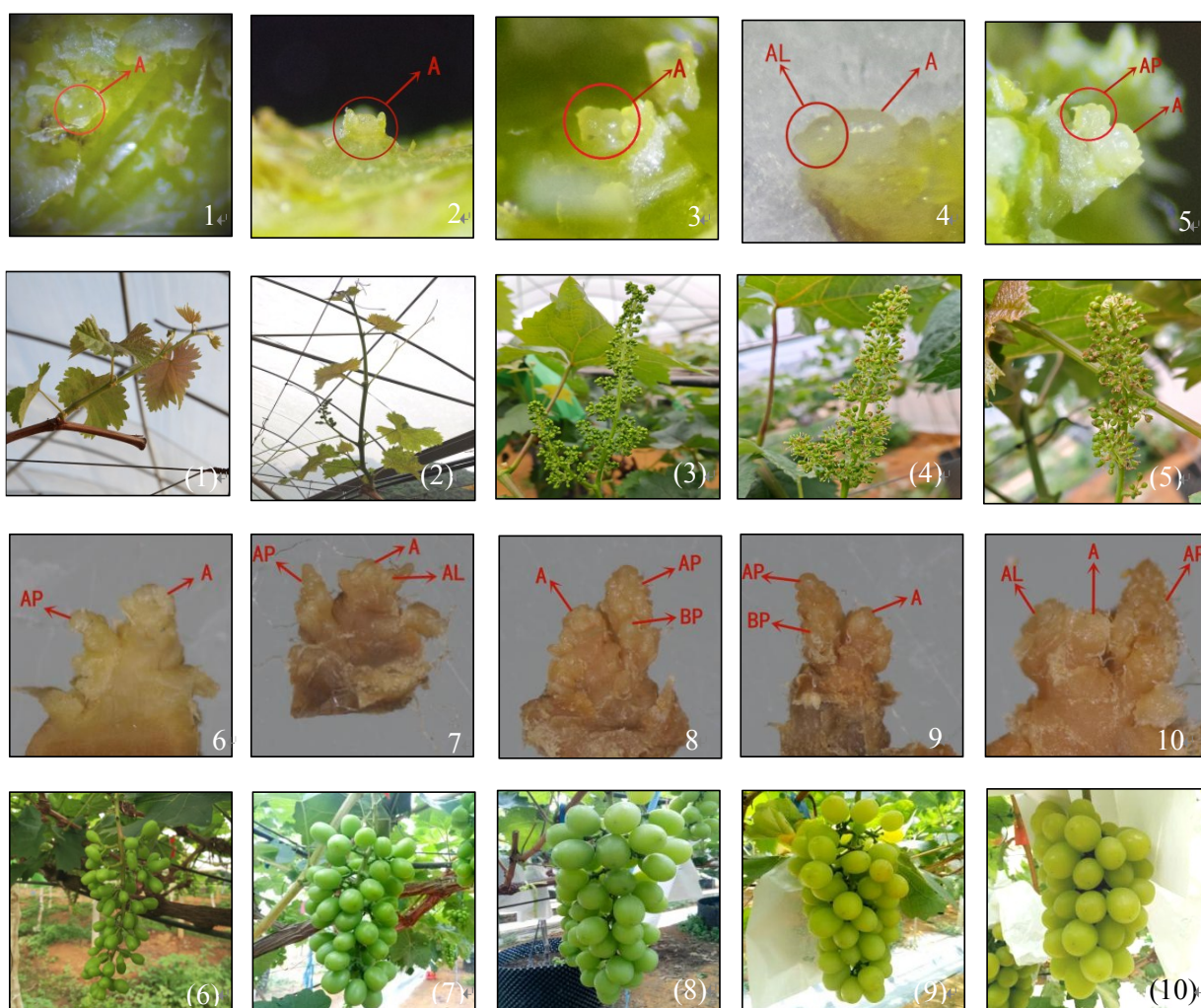
1. 叶芽期；2. 平顶期；3. 二分体时期；4. 始原基分化期；5. 花序原基分化期；6. 花序原基分化期后 20 d；7. 花序原基分化期后 40 d；8. 花序原基分化期后 60 d；9. 花序原基分化期后 80 d；10. 花序原基分化期后 100 d.（1）新梢 5 片展叶期；（2）新梢 6 片展叶期；（3）花穗分离期；（4）盛花期；（5）末花期；（6）幼果期；（7）果实膨大期；（8）果实软化期；（9）果实成熟期；（10）果实采收期。A. 生长点；AL. 始原基；AP. 花序原基；BP. 分支原基。

1. Leaf bud stage; 2. Flat top stage; 3. Dichotomy stage; 4. Anlagen differentiation stage; 5. Inflorescence primordium differentiation stage; 6. Stage of 20 days after inflorescence primordium differentiation; 7. Stage of 40 days after inflorescence primordium differentiation; 8. Stage of 60 days after inflorescence primordium differentiation; 9. Stage of 80 days after inflorescence primordium differentiation; 10. Stage of 100 days after inflorescence primordium differentiation. (1) Stage of 5 leaves spread; (2) Stage of 6 leaves spread; (3) Flower spikes segregation stage; (4) Flower full-bloom stage; (5) Flowering ending stage; (6) Young fruit stage; (7) Fruit enlargement stage; (8) Fruit softening stage; (9) Fruit ripening stage; (10) Fruit harvest period. A. Apex; AL. Anlagen; AP. Inflorescence primordium; BP. Branching primordium.

图 1　阳光玫瑰葡萄各采样期冬芽分化形态结构及其对应的物候期

Fig. 1　The morphology structure of winter bud differentiation of Shine Muscat grape in each sampling period and its corresponding phenological period

2.4　阳光玫瑰葡萄冬芽花芽分化过程中芽内9个成花关键基因表达量的变化

FT、SOC1 及 LEAFY 是成花调控重要整合因子。本研究结果（图4）表明，成花诱导关键基因 VvFT 和 VvSOC1 在阳光玫瑰葡萄冬芽花芽形态分化起始阶段表达量较高。在花芽分化过程中

VvFT基因表达水平变化较大，在平顶期达到最高值后显著下调，在花序原基分化后 20 d 出现第二次波峰后下降，在花序原基分化后 40 d 时最低；VvSOC1 基因表达水平在花芽分化开始后显著上调，在二分体时期达到最大值后显著下降，在花序原基分化后 40 d 后一直处于较低水平；VvLFY基因

表 2 阳光玫瑰葡萄各采样期的冬芽花芽分化阶段与其相应的物候期

Table 2 The differentiation stage of winter buds of Shine Muscat grape in each sampling period and its corresponding phenological period

采样批次 Sampling batch	花芽分化阶段 Differentiation stage	物候期 Phenological period of sampling
1	叶芽期 Leaf bud stage	新梢5片展叶期 Stage of 5 leaves spread
2	平顶期 Flat top stage	新梢6片展叶期 Stage of 6 leaves spread
3	二分体期 Dichotomy stage	花穗分离期 Flower spikes segregation stage
4	始原基分化期 Anlagen differentiation stage	盛花期 Flower full-bloom stage
5	花序原基分化期 Inflorescence primordium differentiation stage	末花期 Flowering ending stage
6	花序原基分化期后20 d Stage of 20 days after inflorescence primordium differentiation	幼果期 Young fruit stage
7	花序原基分化期后40 d Stage of 40 days after inflorescence primordium differentiation	果实膨大期 Fruit enlargement stage
8	花序原基分化期后60 d Stage of 60 days after inflorescence primordium differentiation	果实软化期 Fruit softening stage
9	花序原基分化期后80 d Stage of 80 days after inflorescence primordium differentiation	果实成熟期 Fruit ripening stage
10	花序原基分化期后100 d Stage of 100 days after inflorescence primordium differentiation	果实采收期 Fruit harvest period
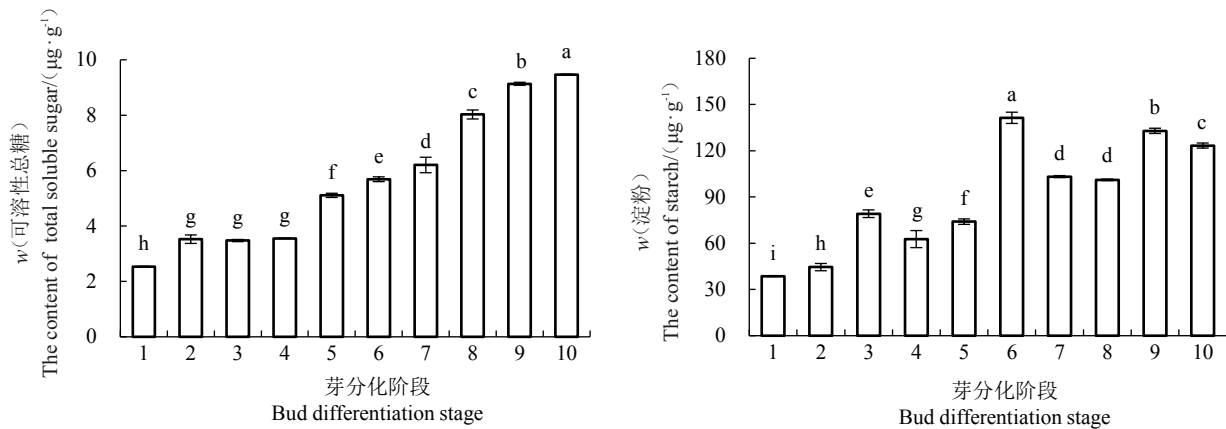
1. 叶芽期；2. 平顶期；3. 二分体时期；4. 始原基分化期；5. 花序原基分化期；6. 花序原基分化后 20 d；7. 花序原基分化后 40 d；8. 花序原基分化后 60 d；9. 花序原基分化后 80 d；10. 花序原基分化后 100 d。不同小写字母表示差异显著（$p<0.05$）。下同。

1. Leaf bud stage; 2. Flat top stage; 3. Dichotomy stage; 4. Anlagen differentiation stage; 5. Inflorescence primordium differentiation stage; 6. stage of 20 days after Inflorescence primordium differentiation; 7. stage of 40 days after Inflorescence primordium differentiation; 8. stage of 60 days after Inflorescence primordium differentiation; 9. stage of 80 days after Inflorescence primordium differentiation; 10.stage of 100 days after Inflorescence primordium differentiation; Different small letters indicate significant difference at $p<0.05$. The same below.

图 2 阳光玫瑰葡萄冬芽花芽分化过程中可溶性总糖和淀粉的含量

Fig. 2 The total soluble sugar and starch content during flower bud differentiation of Shine Muscat grape

的表达水平在花芽分化前期较低，在花序原基分化期出现一次小波峰，其后在花序原基分化后80 d再次出现一次大的波峰。以上结果表明，*VvFT* 和 *VvSOC1* 基因主要参与调控葡萄花芽形态分化起始和始原基分化，*VvLFY* 基因主要参与始原基分化及花序各级穗轴分化。*VvFT* 基因在平顶期及 *VvSOC1* 在二分体期的高表达利于促进花芽进入始原基分化阶段。

AP1 及其同源旁系基因 *FUL* 为花分生组织特征基因。*VvAP1* 基因在阳光玫瑰葡萄冬芽花芽分化前期表达水平较低，在花序原基分化期显著上升，此后其表达水平呈波动变化，分别在花序原基分化后20 d和100 d两次达到最高值，*VvAP1* 的高表达可能有利于促进花序各级穗轴分化和发育。*VvFUL* 基因在花芽分化前期表达水平较低，在花序原基分化期表达上升，其后呈现波动变化，至花序原基分化后80 d
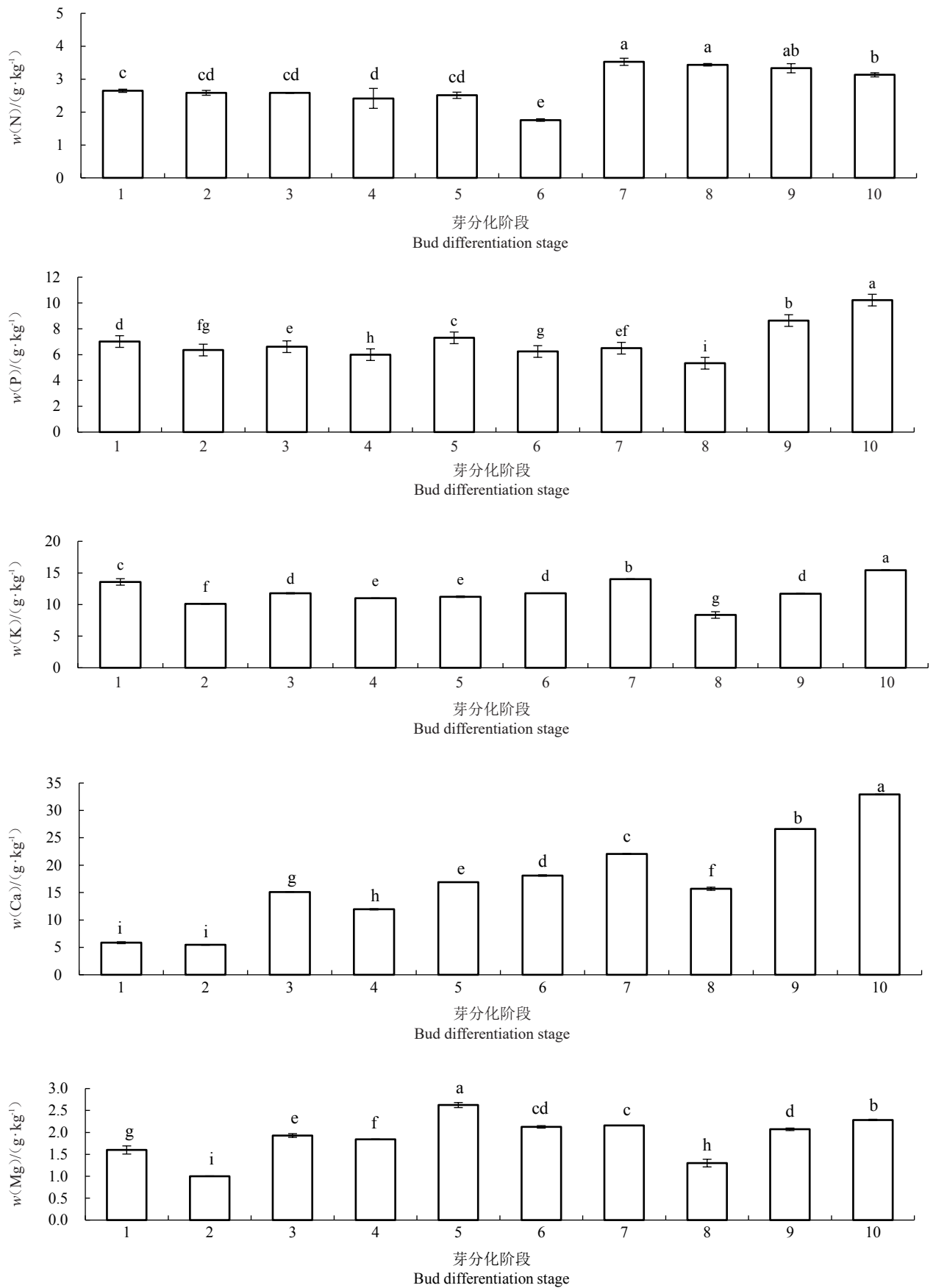
图 3　阳光玫瑰葡萄冬芽花芽分化过程中 N、P、K、Ca、Mg 元素的含量

Fig. 3　The total N, P, K, Ca and Mg content during flower bud differentiation of Shine Muscat grape
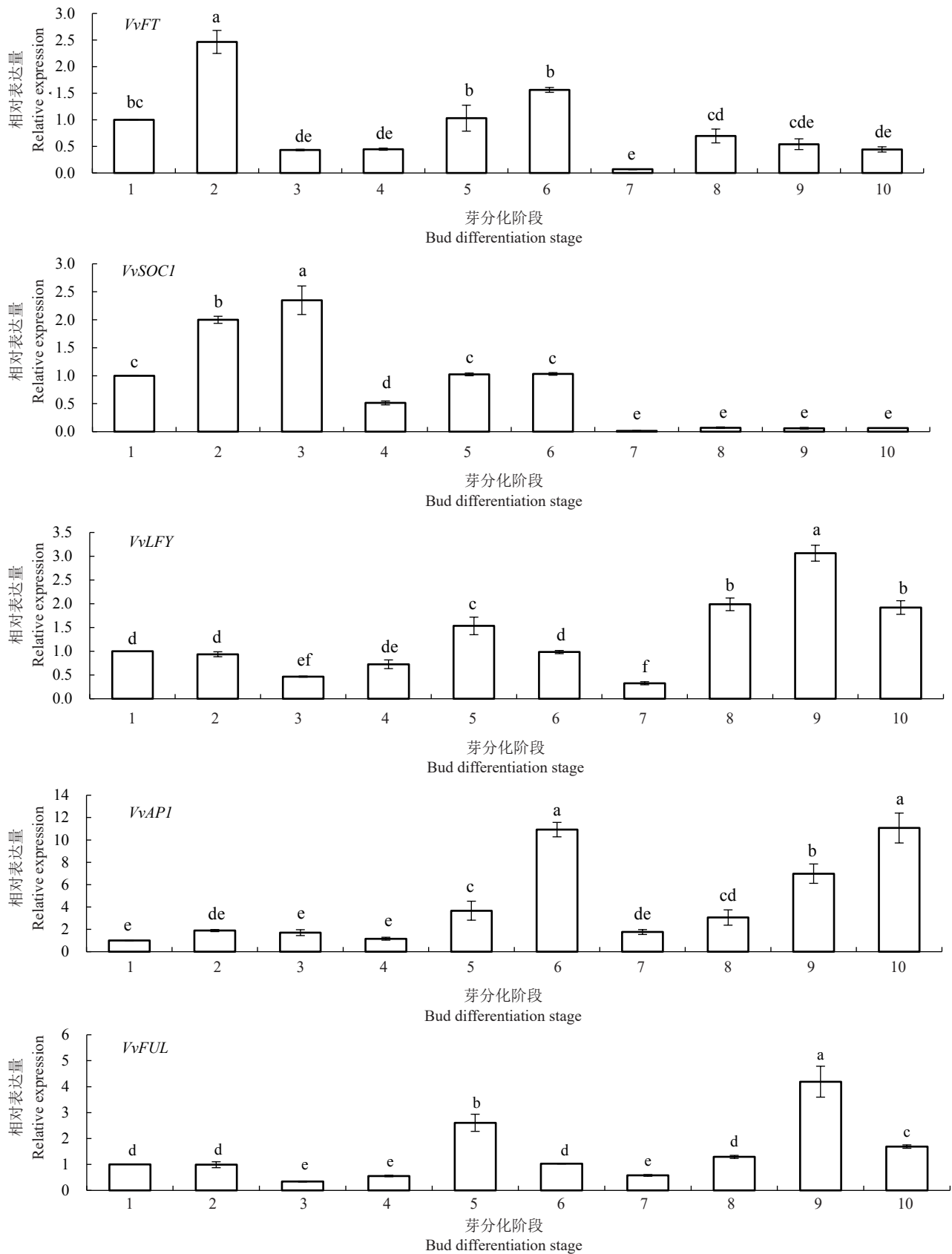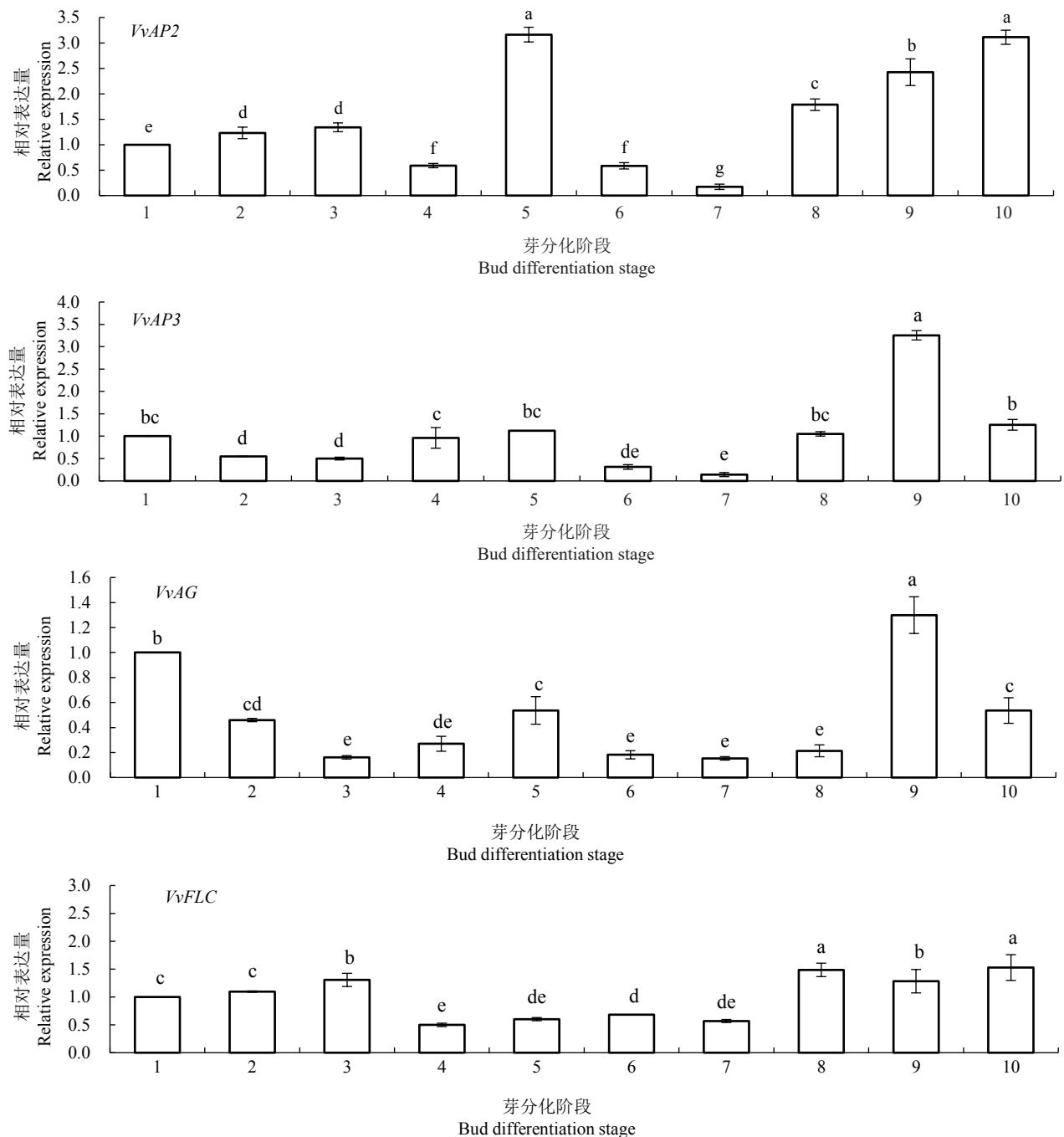
图 4　阳光玫瑰葡萄冬芽花芽分化过程中 *VvFT*、*VvSOC1*、*VvLFY*、*VvAP1*、*VvFUL*、*VvAP2*、*VvAP3*、
VvAG、*VvFLC* 基因的表达趋势

Fig. 4　The variation of expression levels of *VvFT, VvSOC1, VvLFY, VvAP1, VvFUL, VvAP2, VvAP3, VvAG* and *VvLFY* in the flower bud differentiation process of winter buds of Shine Muscat grape

续图　Continued Figure

时表达水平达最高,表明 *VvFUL* 基因主要参与花序原基及花序各级穗轴分化。

　　AP2、*AP3* 和 *AG* 是花器官特征基因。*VvAP2* 基因在花序原基分化期达到最高表达水平后显著下调,在花序原基分化后 40 d 至 100 d 期间呈显著上升趋势,*VvAP2* 基因在花序原基分化期的高表达利于促进花序原基及花序各级穗轴分化。*VvAP3* 和 *VvAG* 基因在冬芽花芽分化过程中的表达趋势一致,*VvAP3* 和 *VvAG* 基因在花芽分化起始时表达显

著下调,在花序原基分化后 80 d 时表达水平最高,表明 *VvAP3* 和 *VvAG* 基因与花序原基及其各级穗轴分化相关。

　　FLC 是开花抑制因子。在阳光玫瑰葡萄花芽分化过程中,*VvFLC* 基因在始原基分化期至花序原基分化期后 40 d 的表达水平显著低于其他时期,在花序原基分化 60~100 d 的表达保持在相对较高水平,表明 *VvFLC* 基因可能参与抑制葡萄花芽分化起始及花序各级穗轴分化。

3 讨 论

葡萄花芽分化进程与树体生长发育期相对应，不同葡萄品种间花芽分化阶段对应的树体物候期不同。研究表明，维多利亚葡萄冬芽在新梢盛花期开始花芽分化，红宝石和红地球葡萄冬芽在末花期才开始花芽分化[36-38]。夏黑葡萄冬芽在新梢长至7~8片叶时开始花芽分化，坐果期才进行花序原基分化[33,39-40]。本研究发现，阳光玫瑰葡萄在新梢6片展叶期时开始花芽分化，在末花期进行花序原基分化，可见阳光玫瑰葡萄冬芽花芽分化进程早于上述葡萄品种，这可能与品种特性及产区气候条件有关。

碳水化合物是葡萄花芽分化重要的能量和营养物质，影响着葡萄成花的全过程[41]。吴月燕等[9]研究发现，无核鸡心葡萄叶片中淀粉和糖类含量与花芽分化呈正相关，充足的糖和淀粉可以促进葡萄花芽分化。赵静等[42]研究夏黑葡萄冬芽成花过程中叶片碳氮物质代谢变化，发现叶片中可溶性总糖与淀粉含量增加可以缩短夏黑花芽分化进程。本研究中，阳光玫瑰葡萄叶片中可溶性总糖含量从平顶期开始显著升高，花芽分化过程中可溶性总糖含量不断升高。葡萄叶片中淀粉含量也从平顶期开始显著升高，在花序原基分化期后20 d时达到最大值，花序原基分化期后淀粉处于相对较高水平，与林玲[33]的研究结论一致。以上结果表明，可溶性总糖和淀粉含量增加促进了阳光玫瑰葡萄花芽分化起始，较高水平可溶性总糖与淀粉有利于葡萄花序原基及其各级穗轴分化。

矿质元素是植物生长发育过程中的重要营养成分，与其生长各个阶段息息相关。研究发现，N含量影响着葡萄花序原基的形成和发育，缺N使得葡萄花序原基及花序的数量减少[10]。成花诱导及花序分化需要P的供应，P的缺失将会影响葡萄成花[11,43]。本研究中，在花序原基分化期后20 d时叶片中全N含量显著降低，分析其原因可能是此时正处于夏果幼果期，且新梢还在继续生长，葡萄果实和叶片生长发育过程对N需求量较大，导致此时叶片中N含量显著下降，此时，及时补充氮源利于形成强大的光合叶幕，以满足树体生长、果实发育及冬芽花芽分化的氮素营养需求。赵文东等[11]研究发现，巨峰葡萄叶片P含量在花芽分化初期较低，在花芽分化过程中呈先下降后上升再下降趋势。李双岑[13]研究发现，

在红地球葡萄花芽分化的过程中芽内P、K含量在花序原基分化时迅速升高，Ca、Mg和N含量随花芽分化进程不断升高。本研究中，叶片中P、K和Mg含量在平顶期时显著下降，此后均出现两次波峰，P和Mg含量在花序原基分化期达到第一个波峰，K在花序原基分化期后40 d达到第一个波峰，P、K和Mg均在花序原基分化期后100 d时达到第二个波峰，这与赵文东等[11]及李双岑[13]的研究结果基本一致，叶片中P、K和Mg含量在平顶期时显著下降，可能是由于花芽分化起始对P、K和Mg需求较大，此时易移动元素P、K和Mg从叶片中移动至芽内供应冬芽需求，使得叶片含量显著降低。本研究中，叶片Ca含量随花芽分化进程而整体不断增加，Ca元素的积累有助于花芽分化，该结论与李双岑[13]一致。本研究发现，P、K、Ca和Mg含量在花序原基分化期后60 d时均显著下降，此时正处于果实软化期，推测冬芽内矿质元素含量变化一定程度上受果实发育时营养竞争影响，生产上应该在夏果果实软化期前予以磷、钾、钙、镁肥的补充，以保证果实发育及花序原基分化正常进行。

葡萄花芽分化过程受内部基因的调控。前人研究发现，在葡萄成花诱导至花序原基分化过程中，基因FT、SOC1、LEAFY、AP1、FUL和FLC表达上调，参与调控成花诱导、始原基和花序原基分化。FT基因是成花时间的关键决定基因[17,44]，正调控其下游基因SOC1的表达[45-46]。葡萄中VvFT基因在花分生组织诱导形成过程中表达上调[35]。VvSOC1基因在葡萄花序原基和苞片中的表达处于较高水平[22]。花分生组织特征基因LEAFY可通过激活AP1基因来促进花序向花分生组织转变[21,23]。VvLEAFY基因在葡萄花序原基发育过程中的表达处于较高水平[24]。VvAP1与VvFUL基因在葡萄花芽分化早期的分生组织中就有表达[25]。FLC基因在花序原基诱导和分化过程中表达上调[47]，葡萄中VvFLC基因会抑制VvFT和VvSOC1的表达，负调控花序原基的形成[48]。本研究中，9个成花关键基因在成花诱导至花序原基分化过程中的表达情况与前人研究基本一致[31,48-50]。在阳光玫瑰葡萄冬芽分化平顶期即花芽形态分化起始时，基因VvFT、VvSOC1、VvAP1和VvAP2的表达显著上调；在花序原基分化期，基因VvFT、VvSOC1、VvLFY、VvAP1、VvFUL、VvAP2、VvAP3和VvAG表达均显著上调；VvFLC基因在始原

基分化期前表达水平相对较高，在始原基分化期表达显著下调。以上结果表明，基因*VvFT*和*VvSOC1*参与并促进了形态分化起始及始原基和花序原基分化，而*VvFLC*基因可能会抑制花芽进入形态分化阶段，基因*VvAP1*、*VvAP2*、*VvLFY*、*VvFUL*、*VvAP3*和*VvAG*可能均参与诱导花序原基分化。前人对葡萄花芽分化相关分子研究多集中在成花诱导至花序原基分化阶段，然而在花序原基分化至花序各级穗轴分化过程中的研究还未见详细报道。笔者在本试验中探究了这9个成花关键基因在花序原基分化至花序各级穗轴分化过程中的表达情况，研究发现，基因*VvFT*和*VvSOC1*在花序原基分化后的表达水平相对较低，基因*VvLFY*、*VvFUL*、*VvAP3*和*VvAG*在花序原基分化期后80 d表达显著上调并达到表达高峰。基因*VvAP1*和*VvAP2*在花序原基分化期后20 d之后表达持续上调，在花序原基分化期后100 d时达表达波峰。*VvFLC*基因在花序原基分化期后60 d之后表达水平相对较高。以上结果表明，在花序原基分化期后基因*VvFT*和*VvSOC1*对花序各级穗轴的分化及发育的调控减弱，基因*VvLFY*、*VvAP1*、*VvFUL*、*VvAP2*、*VvAP3*和*VvAG*可能均参与促进花序各级穗轴的分化及发育，而*VvFLC*则可能抑制花序各级穗轴的分化。

4　结　论

研究探明了广西南宁地区阳光玫瑰葡萄生长期冬芽花芽分化进程，确定了阳光玫瑰葡萄花芽分化各阶段对应的物候期，发现南宁地区阳光玫瑰葡萄花芽分化进程比多数已报道的葡萄品种开始得早。在进入花序各级穗轴发育期后叶片可溶性总糖和淀粉含量较高，叶片P、K、Ca、Mg元素含量均在花序原基分化期后60 d时显著降低。阳光玫瑰葡萄冬芽在花序原基分化期后对有机营养和主要矿质元素的需求增高，生产上在阳光玫瑰葡萄果实膨大期和软化期前后应注意适当补充磷、钾、钙、镁肥，以促进果实发育及花序各级穗轴分化正常进行。*VvFT*和*VvSOC1*基因参与诱导始原基及花序原基分化，*VvLFY*、*VvAP1*、*VvFUL*、*VvAP2*、*VvAP3*和*VvAG*基因可能均参与了花序原基分化及花序各级穗轴的分化及发育。*VvFLC*基因在始原基前及花序原基分化后期的高表达可能会抑制始原基及花序各级穗轴的分化。

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