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石榴*SUT*基因家族鉴定及其在籽粒发育过程中的表达分析

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摘 要:【目的】对石榴*SUT*家族基因成员进行全基因组鉴定,并分析*SUT*家族基因成员的结构域特征、启动子序列、生化特征、进化关系和表达特性,为深入研究该基因作用及其分子调控机制提供参考。**【方法】**利用同源比对及HMMER模型鉴定石榴*SUT*基因家族成员;利用邻近法和基因结构分析解析石榴*SUT*基因及其启动子序列的系统发生关系。**【结果】**共鉴定了10个石榴*SUT*基因,可以分为3大类;石榴*SUT*基因及其启动子区的GC含量都明显低于其在拟南芥和水稻中的含量;石榴*SUT*家族基因GC含量远高于启动子区的GC含量;石榴*SUT*基因的序列长度与GC含量呈明显正相关;石榴*SUT*基因启动子区含有10种MYB元件;此外,4个石榴*SUT*基因(*PgL0328370.1*、*PgL0099690.1*、*PgL0145810.1*和*PgL0145770.1*)在籽粒发育过程中发生了差异表达。**【结论】***SUT*基因在进化过程中其序列和基因结构相对保守,而其启动子序列在进化过程中变化较大,*SUT*基因的表达量变化与籽粒发育显著相关。

关键词:石榴;*SUT*;基因家族;籽粒;表达分析

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Identification of *SUT* genes and their expression models during the development of seed in pomegranate

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Abstract:【Objective】Photosynthetically produced sugar, principally sucrose, is moved from source leaves to support growth of, and carbon storage by, heterotrophic sink organs. Membrane proteins play pivotal roles in mediating sucrose transport within plants. Pomegranate (*Punica granatum* L.), native to central Asia, is an ancient medicinal fruit crop grown worldwide, with considerable economic value. Pomegranate is well famed as a highly valuable fruit with high nutritive and medical attributes. The pomegranate's flower, fruit peel, aril (juice), and seeds, are useful for the prevention and treatment of a wide range of diseases. Their functional advantages have dramatically stimulated the market demand, which has opened the avenue for the breeding programs in pomegranate. Mapping genes related to horticulturally important traits is helpful to the molecular marker-assisted (MAS) breeding. To data, *SUTs* activities had been proved to have a major impact upon regulating many plant developmental processes, such as pollen germination, flowering, tuberization, restraining plant growth, fruit size reduction, seed development, biomass partitioning, plant growth rates, crop yields and ethylene biosynthesis. Here, the objective of this study was to identify the genomic *SUT* genes, and analyze their gene structure, promoter, phylogenetic relationship and expression models. It will provide the reference for further research on the function and molecular regulation of *SUT* family in pomegranate.【Methods】Blastp and HMMER

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model were performed to identify the *SUT* genes; The phylogenetic relationships of *SUTs* and their promoters were analyzed by adjacent method and gene structure analysis.【Results】There were nine sucrose transporter genes described in *Arabidopsis*, whereas the rice genome contained five *SUT* genes. In the study, a total of 10 *SUT* genes were detected on the Chr1, Chr3, Chr4, Chr5, Chr6 and Chr7 in pomegranate, whose number was more than that of *Arabidopsis* and rice. The average sequence length of *SUT* genes was 4 406.7 bp. The average content of A, C, G and T for *SUT* genes was 28.50%, 21.57%, 22.77% and 27.14%, respectively. The average content of GC for *SUT* genes was 44.35%, which was comparable to that of ‘Tunisia’ genome. Additionally, the mean length of *SUT* genes was significantly related to that of GC content with the relationship coefficient being 0.87 at $p < 0.01$ level. The length of SUT proteins varied from 92 aa to 1 251 aa, and the molecular weight of *SUT* proteins changed from 10 260.37 to 142 472.24 D. Relationship analysis indicated that the length of *SUT* proteins was significantly negatively related to the content of His, but positively related to the content of Met at $p < 0.05$, and the relationship coefficients were −0.67 and 0.65, respectively. Increasing availability of molecular information could provide new opportunities to detect physiological roles for, and regulation of, sucrose transporters. In the study, the phylogenetic analysis indicated that these *SUT* genes were divided into three groups, named group1, group2 and group3. The group1, group2 and group3 contained four, one and five *SUT* genes, respectively. The GC contents of *SUT* genes and their promoters in pomegranate were significantly lower than those of *Arabidopsis thaliana* and *Oryza sativa*. The GC contents of *SUT* genes were significantly higher than those of their promoters in pomegranate. There was a significant relationship between the GC contents of *SUT* genes and their length. We also detected ten MYB elements in the promoter domain for the *SUT* genes. The MYB elements mainly contained MYB2AT, MYB2CONSENSUSAT, MYBCORE, MYBCOREATCYCB1, MYBGAHV, MYBST1, MYBPLANT, MYBPZM, MYB1AT and MYB1LEPR. *PgL0145810.1*, *PgL0237030.1* and *PgL0181920.1* all contained more than 20 MYB elements, while *PgL0099690.1* only contained seven elements. In addition, four *SUT* genes containing *PgL0328370.1, PgL0099690.1, PgL0145810.1* and *PgL0145770.1* were detected to differentially express during the development of the seed by previous transcriptomic sequencing in pomegranate. *PgL0099690.1* was down-regulated after 120 flowering days in ‘Sanbai’ seeds compared to after 60 flowering days. Both *PgL0145810.1* and *PgL0145770.1* were down-regulated in ‘Sanbai’ seeds after 120 flowering days compared to after 60 flowering days. Conversely, *PgL0328370.1* was up-regulated after 120 flowering days in ‘Sanbai’ or ‘Tunisia’ seeds compared to after 60 flowering days. For the different cultivars, *PgL0145770.1* was down-regulated after 60 flowering days in ‘Tunisia’ seeds compared to that in ‘Sanbai’.【Conclusion】The *SUTs* were encoded by a multi-gene family. The *SUT* family has been characterized in many plant species. The molecular characteristics, structures, and phylogeny of plant *SUTs* have been well studied and reviewed. Our results indicated that *SUT* genes were relatively conservative in their sequences and gene structures during evolution in pomegranate. However, their promoter sequences changed greatly in evolutionary process; Developing plant embryos depended on nutrition from maternal tissues via the seed coat and endosperm. Sucrose, the major transport form of carbohydrate in plants, was delivered via the phloem to the maternal seed coat and then secreted from the seed coat to feed the embryo. Thus, the expression variation of *SUT* genes regulated the development of the seed in pomegranate. Our findings will provide a foundation for further functional studies on *SUTs* in pomegranate, and contribute to elucidating *SUT* roles in seed development.

Key words: Pomegranate; *SUT*; Gene family; Seed; Expression analysis

石榴 (*Punica granatum* L.) 属于千屈菜科 (Lythraceae)、石榴属 (*Punica*)，是一种古老的落叶灌木或小乔木果树树种，栽培历史悠久[1]。目前，研究学者广泛认可石榴起源于伊朗、阿富汗等中亚地区，后在地中海和中东地区驯化栽培[2]。西汉时期，石榴传入中国并开始广泛栽培种植。截至 2018 年底，中国已形成了以陕西、山东、河南、四川、安徽、云南等地为主的几个主要石榴产区，栽培面积约 12 万 hm^2，年产量高达 120 万 t。石榴是一种重要的经济树种，其叶片可以制茶，果皮能做药材，枝条可当编条，果实具有丰富营养、药用、保健价值[3]，深受世人喜爱。

定位石榴重要农艺性状形成的关键基因，可以为石榴遗传改良提供理论依据。目前，石榴相关遗传学研究主要集中在籽粒硬度[4-7]、果皮色泽[1,8]、物质代谢[9]、花育性[10]等。虽然大量的差异表达基因被报道，然而目前为止并未有基因被克隆。基因家族分析可以快速的筛选某一类基因家族所有的基因成员，进一步结合组学数据及群体定位可以高效的筛选目标基因，是定位候选基因的一种有效手段。Chen 等[11] 鉴定了油菜基因组水平上的几丁质酶基因家族成员，并揭示了其在抗根瘤病中的作用。Xiao 等[12] 筛选了棉花基因组上的 *GhARF* 基因家族成员，结合转录组数据证实 *GhARF2* 和 *GhARF18* 与棉纤维发育相关。

蔗糖是植物合成淀粉的基本原料，也是光合同化产物运输的主要形式。蔗糖在植物体不同器官间转运和分配[13]，很大程度上参与和影响植株正常的生理代谢途径。植物蔗糖转运基因 (sucrose transporter gene, *SUT* genes) 是一类编码蔗糖转运蛋白基因，*SUT* 家族成员序列相对保守，是蔗糖转运途径中的关键因子。同时，*SUT* 家族成员也是联系源库代谢的信号转导元件。当植物体受到外界变化的刺激，如温度、光周期、病原菌等，蔗糖转运子会被精密调控，从而改变其转运活性，以此来适应环境的变化[14-15]。植物上第一个 *SUT* 基因 *SoSUT* 是从菠菜中发现的[16]，之后大量的 *SUT* 基因在水稻[17]、拟南芥[18-20]等模式植物以及梨属等木本植物[21]中被报道。拟南芥作为双子叶植物的模式植物，共有 9 个蔗糖转运基因（*AtSUT*1~9），其表达量变化决定着花器官发育、脱落酸响应、下胚轴伸长等重要性状。Li 等[22]从油菜种子产量数量性状基因座上定位了一个 *SUT* 基因，通过关联分析证实该基因与有效分枝数、角果数和千粒重显著相关。然而，相应的 *SUT* 基因家族成员鉴定及其功能分析在石榴基因组研究中还没有被报道。本研究旨在筛选石榴全基因组中 *SUT* 基因家族成员，结合转录组数据分析其在籽粒发育过程中的表达模式，为下一步 *SUT* 基因的功能验证与利用提供参考。

1　材料和方法

1.1　材料

石榴的全基因组序列为‘突尼斯’基因组序列[23]；拟南芥 *SUT* 基因序列和蛋白序列下载自 TAIR (https://www.arabidopsis.org/)。‘突尼斯’和‘三白’石榴品种定植于中国农业科学院郑州果树研究所试验场。

1.2　*SUT*候选基因的鉴定

SUT 基因家族含有 GPH_sucrose (TIGR01301) 结构域，在 TIGRFAMS (http://tigrfams.jcvi.org/cgi- bin/index.cgi) 数据库中下载 TIGR01301 的种子序列，用 ClustalW[24]进行序列比对，将比对结果构建一个 *SUT* 蛋白结构特异的隐马尔科夫模型(HMM)[25]，并用该模型检索石榴蛋白数据库，然后将得到的石榴 *SUT* 蛋白序列用 ClustalW2 进行比对，重新构建一个 HMM 模型，再一次检索石榴蛋白数据库，直至穷尽整个石榴数据库。用 TIGR01301 的种子序列做目标序列，对已经搜索到的石榴蛋白序列进行 Blastp[26]比对分析，以 E 值小于 10~15 做筛选条件，最终得到 *SUT* 基因家族所有成员。

1.3　*SUT*基因家族的蛋白序列聚类分析、基因结构及启动子分析

采用 ClustalW 进行多序列比对后，应用 MEGA5.0 [27] Neighbor-Joining 法构建系统发生树，bootstrap 值设为 1 000，对 *SUT* 的蛋白序列及启动子序列进行聚类分析。利用获得的 *SUT* 成员基因，提取对应的 gff 文件，获得各基因内含子数目以及基因结构。提取基因上游 1.5 kb 的序列作为启动子序列，并用在线工具 PlantCARE（http://bioinformatics.psb.ugent.be/webtools/plantcare/html/）做顺式作用元件预测。利用在线工具 GSDS 2.0 (http://gsds.cbi.pku.edu.cn/) 绘出基因结构图及顺式作用结构图。

1.4 *SUT*基因及其蛋白序列组成成分分析

用 BioEdit 软件(https://bioedit.software.informer.com)分析石榴 *SUT* 基因及其上游序列的核酸组成成分，并计算其 GC 含量。同样的软件用来分析 *SUT* 蛋白的氨基酸组成成分及分子重量(Molecular Weight, M/W)。

1.5 相关性分析

SUT 核酸组分与基因长度及蛋白组分与氨基酸长度之间的相关性分析，氨基酸平均含量间差异分析均采用 SPSS19.0 (IBM Corp., Armonk, NY, USA)软件。

1.6 实时荧光定量PCR分析

利用 RNAprep Pure Plant Kit 试剂盒（天根公司）提取石榴籽粒样品 RNA，经琼脂糖凝胶电泳和紫外分光光度计检测后，选取质量合格的 RNA 样品，使用 NCBI Primer Blast 软件(https://www.ncbi.nlm.nih.gov/tools/primer-blast/) 设计引物（表 1），使用荧光定量 PCR 仪（Roche 480，SYBR GreenI）进行实时荧光 RT-PCR 分析，设置 3 次重复，相对表达量的计算采用 $2^{-\triangle\triangle Ct}$ 法[28]，表达量热图采用 R 中“pheatmap” (https://CRAN.R- project.org/package=pheatmap) 绘制。

表1 实时荧光定量PCR分析引物序列

Table 1 Primers used for qRT-PCR

基因名称 Gene name	引物 Primer	引物序列 (5’-3’)sequence
PgL0328370.1	上引物 Forward primer	GACCCGCAAACAAAGTCACC
	下引物 Reverse primer	GTTCCGACCGGATCTAGCAG
PgL0099690.1	上引物 Forward primer	ACCACTGCTCTCATGTTCCG
	下引物 Reverse primer	TCCGTGAACCCTAAAGTGGC
PgL0145810.1	上引物 Forward primer	CTTTCTAGAATGGAGGTTGAATCAGTCAA
	下引物 Reverse primer	CTTGAGCTCTTAATTGGAAATGGTCAT
PgL0145770.1	上引物 Forward primer	CTTTCTAGAATGGAAATGGAGAACGGAAT
	下引物 Reverse primer	CTTGAGCTCTCAGTGTCCACCTGCAA

2 结果与分析

2.1 石榴*SUTs*家族基因及其启动子序列分析

通过比对分析，在‘突尼斯’石榴基因组数据库中共获取到 *SUT* 基因家族的 10 个成员，分布在 Chr1、Chr3、Chr4、Chr5、Chr6 和 Chr7 上 (表 2)。其中，Chr3 和 Chr6 含有的 *SUT* 基因家族成员数量最多，均为 3 个。结果分析表明物种内不同 *SUT* 基因的长度变化较大，石榴 *SUT* 基因序列的平均长度为 4 406.7 bp。石榴 *SUT* 家族基因最长与最短序列相

表2 石榴*SUT*家族基因及其核苷酸组成

Table 2 Nucleotide composition of *SUT* genes in pomegranate

基因名称 Gene name	染色体 Chr	起始位置 Start	终止位置 End	基因长度 Gene length/bp	腺嘌呤占比 A/%	胞嘧啶占比 C/%	鸟嘌呤占比 G/%	胸腺嘧啶占比 T/%	(胞嘧啶+鸟嘌呤)占比 (C+G)/%
PgL0099690.1	Chr1	2954359	2961954	7 596	29.30	17.83	22.80	30.07	40.63
PgL0145770.1	Chr3	327678	330169	2 492	28.18	22.84	24.97	24.01	47.81
PgL0145810.1	Chr3	341247	345377	4 131	31.99	19.61	21.33	27.07	40.94
PgL0181920.1	Chr3	36608641	36613106	4 466	34.13	21.59	19.89	24.39	41.48
PgL0233780.1	Chr4	39683636	39690941	7 306	30.44	17.63	21.75	30.17	39.38
PgL0237030.1	Chr5	1480436	1488449	8 014	30.38	21.30	17.97	30.35	39.27
PgL0281820.1	Chr6	13470881	13476350	5 470	29.77	17.15	23.66	29.42	40.81
PgL0281800.1	Chr6	13476502	13477048	547	26.01	24.36	25.46	24.18	49.82
PgL0281810.1	Chr6	13470045	13470323	279	20.5	32.01	27.34	20.14	59.35
PgL0328370.1	Chr7	24075849	24079614	3 766	24.33	21.46	22.55	31.66	44.01
平均值 Mean				4 406.7	28.50	21.57	22.77	27.14	44.35

差 7 735 bp。石榴 *SUT* 基因腺嘌呤、胞嘧啶、鸟嘌呤及胸腺嘧啶的平均含量分别为 28.50%、21.57%、22.77%和 27.14%。GC 平均含量为 44.35%，与石榴基因组 GC 含量相当。启动子序列腺嘌呤及胸腺嘧啶的平均含量高于基因序列，分别为 30.51%和 30.71% (表 3)。然而，启动子区序列的胞嘧啶、鸟嘌呤及 GC 平均含量均低于基因组序列，分别为 19.17%、19.62%和 38.79% (表 3)。相关分析表明石榴 *SUT* 基因长度与其 GC 含量显著相关 ($p < 0.01$)，相关系数为 0.87。

表3　石榴*SUT*家族基因启动子及其核苷酸组成

Table 3　Nucleotide composition of *SUT* promoters in pomegranate

基因名称 Gene name	腺嘌呤占比 A/%	胞嘧啶占比 C/%	鸟嘌呤占比 G/%	胸腺嘧啶占比 T/%	（胞嘧啶+鸟嘌呤）占比 (C+G)/%
PgL0099690.1	33.53	17.93	15.53	33.00	33.47
PgL0145770.1	34.53	15.93	19.13	30.40	35.07
PgL0145810.1	29.93	16.47	22.60	31.00	39.07
PgL0181920.1	28.13	23.27	23.80	24.80	47.07
PgL0233780.1	26.93	21.93	21.40	29.73	43.33
PgL0237030.1	29.67	22.40	20.13	27.80	42.53
PgL0281820.1	28.67	20.67	20.80	29.87	41.47
PgL0281800.1	30.20	17.20	24.27	28.33	41.47
PgL0281810.1	34.00	16.00	11.93	38.07	27.93
PgL0328370.1	29.47	19.87	16.60	34.07	36.47
平均值 Mean	30.51	19.17	19.62	30.71	38.79

2.2　石榴*SUT*蛋白及其氨基酸组成分析

表 4 为石榴 *SUT* 蛋白及其氨基酸组成分析，蛋白序列长度变异为 92~1 251 aa，分子质量变异为 10 260.37~142 472.24 Da。几乎所有的 *SUT* 蛋白都包含了人体常见的 20 种氨基酸。然而，*PgL0281820* 和 *PgL0281800* 蛋白组成均不包含色氨酸，*PgL0281810* 蛋白不包含半胱氨酸。*SUT* 蛋白序列的亮氨酸含量平均值最高为 10.69，半胱氨酸含量平均值最低仅为 0.82。*T* 测验分析表明亮氨酸和半胱氨酸平均含量间存在着显著差异（$p < 0.01$）。相关分析表明，石榴 *SUT* 蛋白序列长度与组氨酸含量呈显著负相关（$p < 0.05$），与蛋氨酸呈显著正相关（$p < 0.05$），相关系数分别为−0.67 和 0.65。

2.3　石榴*SUT*基因家族的基因结构和系统发育分析

系统发育树结果分析表明，石榴 *SUT* 基因家族主要分为 3 个大类: group1，group2 和 group3 (图 1)。group1，group2 和 group3 分别含有 4、1 和 5 个 *SUT* 基因。group2 中的 *PgL0237030.1* 含有外显子数目最多。group3 中的 *PgL0099690.1* 和 *PgL0233780.1*，group2 中的 *PgL0237030.1* 以及 group1 中的 *PgL0181920.1* 含有的内含子数目都在 9 以上。其余的 *SUT* 基因含有的内含子数目均在 4 以下。

2.4　石榴*SUT*基因家族的基因启动子和顺式作用元件分析

PLACE 分析显示，所有石榴 *SUT* 基因的启动子区域都含有多个 MYB 元件（图 2）。这些 MYB 元件主要为 MYB2AT、MYB2CONSENSUSAT、MYBCORE、MYBCOREATCYCB1、MYBGAHV、MYBST1、MYBPLANT、MYBPZM、MYB1AT 和 MYB1LEPR 共 10 种类型。*PgL0145810.1*、*PgL0237030.1* 和 *PgL0181920.1* 均含有 20 个以上 MYB 元件，而 *PgL0099690.1* 含有的 MYB 元件最少为 7 个。这些元件都与重要的农艺性状的形成及进化相关，或许是决定 *SUT* 基因的多效性的主要因子。

2.5　石榴*SUT*基因家族的基因在籽粒发育中的表达分析

结合前期转录组数据[4,7]分析结果表明，*SUT* 基因家族中的 *PgL0099690.1*、*PgL0328370.1*、*PgL0145810.1* 和 *PgL0145770.1* 在不同石榴品种籽粒发育中发生了差异表达（图 3）。与石榴开花后 60 d 的籽粒相比，*PgL0099690.1* 在‘三白’石榴开花后 120 d 的籽粒中（SS2）下调表达；*PgL0145810.1* 和 *PgL0145770.1* 在‘三白’和‘突尼斯’石榴开花后 120 d 的籽粒中（TS2）均下调表达；相反，*PgL0328370.1* 不论在‘三白’还是‘突尼斯’石榴开花后 120 d 的籽粒中均上调表达；另外，与‘三白’石榴开花后 60 d 的籽粒（SS1）相比，*PgL0145770.1* 在‘突尼斯’石榴开花后 60 d (TS1) 的籽粒中也下调表达。实时荧光定量 PCR 分析表明（图 4），*PgL0099690.1*、*PgL0328370.1*、*PgL0145810.1* 和 *PgL0145770.1* 在‘三白’和‘突尼斯’石榴不同发育时期籽粒中的表达量变化趋势与转录组结果一致。由此可见，这些 *SUT* 基因可能通过差异表达来调控石榴籽粒发育进程。

表4 *SUT*基因氨基酸组成分析

Table 4 The component of amino acid for *SUT* genes

氨基酸 Amino	蛋白名称 Protein name										平均值 Mean
	PgL0145770	PgL0099690	PgL0145810	PgL0181920	PgL0233780	PgL0237030	PgL0281820	PgL0328370	PgL0281810	PgL0281800	
蛋白长度 Length/bp	498.00	1 236.00	581.00	515.00	1 187.00	1 251.00	92.00	507.00	155.00	289.00	562.92
分子质量 Molecular mass/Da	53 641.48	142 986.14	60 951.37	55 655.52	133 412.98	142 472.24	10 260.37	54 848.93	18 001.63	33 693.48	63 134.94
丙氨酸 Ala	10.24	6.23	12.22	9.51	7.67	7.19	5.43	10.06	3.87	8.30	7.74
半胱氨酸 Cys	1.41	0.65	1.55	1.36	0.84	0.80	1.09	1.38	0.00	0.35	0.82
天冬氨酸 Asp	2.81	5.91	2.93	4.66	4.63	6.00	3.26	3.55	4.52	4.84	4.37
谷氨酸 Glu	2.01	12.14	2.24	3.50	10.53	11.99	5.43	2.56	3.87	16.26	7.56
苯丙氨酸 Phe	6.43	3.16	5.34	5.63	2.78	2.40	5.43	6.11	2.58	1.04	3.71
甘氨酸 Gly	10.64	4.61	8.95	8.35	4.97	4.96	8.70	10.06	6.45	3.11	6.70
组氨酸 His	2.61	1.38	0.69	3.69	1.43	1.44	5.43	1.18	4.52	2.08	2.59
异亮氨酸 Ile	5.82	5.42	4.99	5.05	5.64	5.28	5.43	7.50	5.81	4.84	5.54
赖氨酸 Lys	4.02	11.41	2.58	2.14	10.78	11.75	6.52	1.58	5.81	13.49	7.45
亮氨酸 Leu	13.25	10.28	12.56	10.87	10.78	10.31	10.87	10.65	9.68	9.69	10.69
蛋氨酸 Met	3.01	1.78	2.07	2.52	2.11	2.72	2.17	2.76	3.23	2.08	2.48
天冬酰胺 Asn	3.01	4.13	3.79	5.05	4.80	3.68	5.43	2.96	3.87	3.46	3.96
脯氨酸 Pro	5.42	2.18	7.23	5.05	1.94	2.40	3.26	5.92	5.81	1.38	3.98
谷氨酰胺 Gln	3.01	5.34	2.07	4.08	6.32	4.40	2.17	2.96	2.58	4.84	3.77
精氨酸 Arg	3.21	6.72	3.96	4.08	4.38	5.52	6.52	5.72	7.74	7.96	5.96
丝氨酸 Ser	5.62	6.31	9.12	9.51	7.33	6.95	7.61	6.71	9.68	6.57	7.64
苏氨酸 Thr	3.61	4.37	5.85	3.30	3.71	3.84	3.26	5.33	6.45	2.42	4.25
缬氨酸 Val	9.24	4.77	8.26	8.54	7.33	5.52	9.78	8.28	4.52	4.5	6.65
色氨酸 Trp	2.21	0.65	1.89	1.75	0.34	0.40	0.00	2.76	2.58	0.00	1.26
酪氨酸 Tyr	2.41	2.59	1.72	1.36	1.68	2.48	2.17	1.97	6.45	2.77	2.90

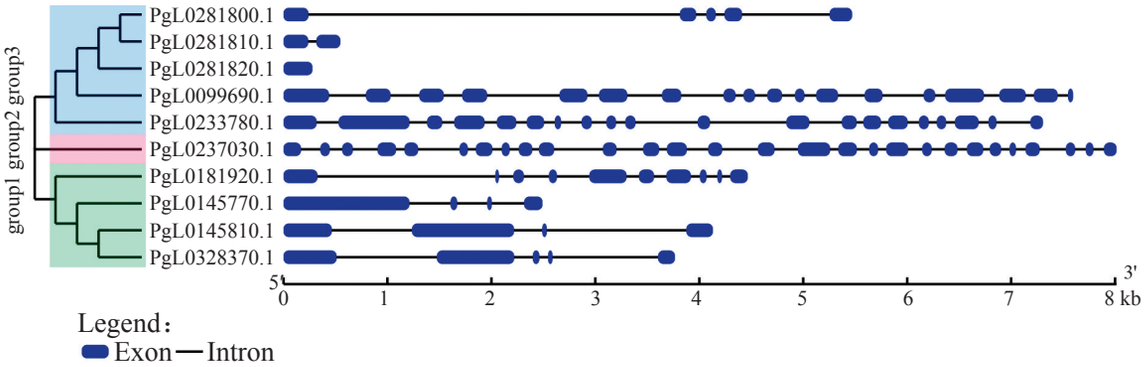


图1　石榴*SUT*基因家族的系统发育和基因结构分析

Fig. 1　Phylogenetic tree of *SUT* genes and their exon/intron structures analysis

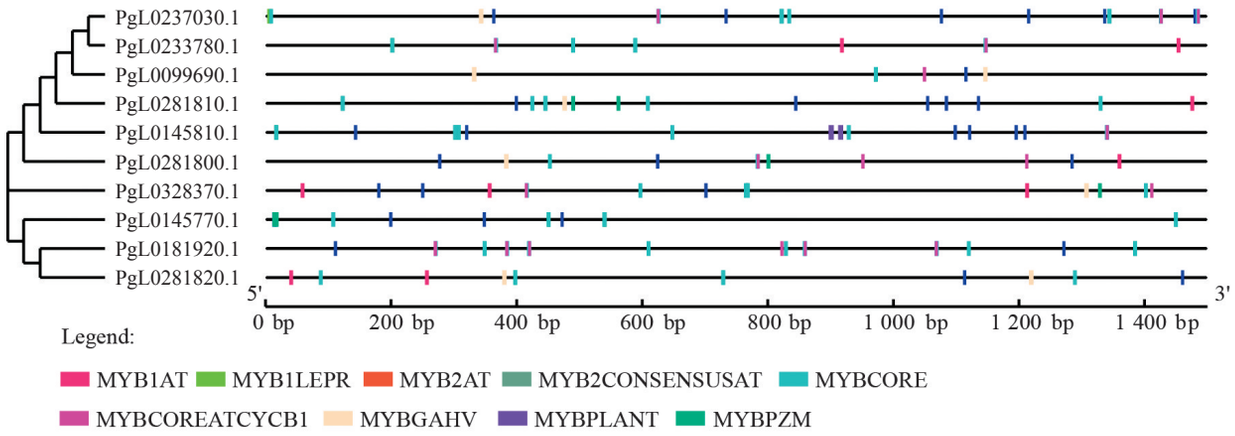
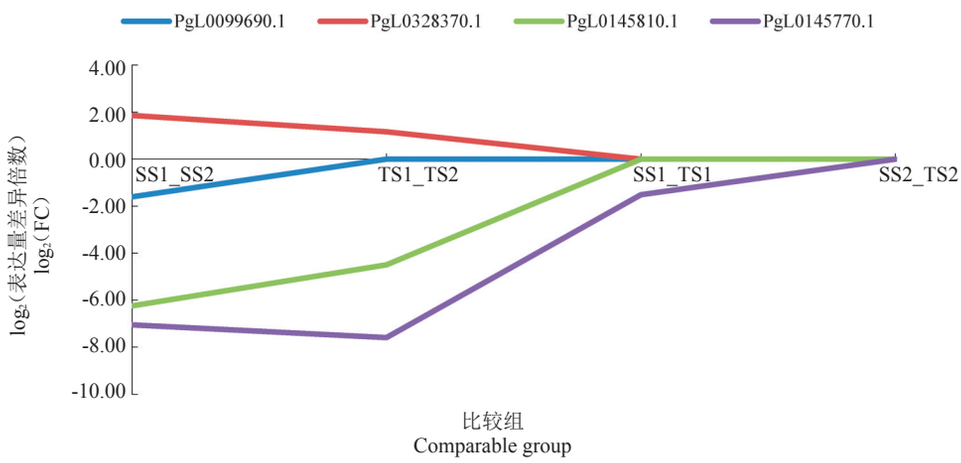


图2　石榴*SUT*基因家族的启动子和顺式作用元件分析

Fig. 2　Promoters of *SUT* genes and their cis-acting element analysis



TS1. 开花后 60 d 突尼斯籽粒; TS2. 开花后 120 d 突尼斯籽粒; SS1. 开花后 60 d 三白籽粒；SS2. 开花后 120 d 三白籽粒；FC. 差异倍数；SS1_SS2,TS1_TS2, SS1_TS1 和 SS2_TS2 为比较组，“_”前的为参考组，“_”后的为试验组。

TS1. Seeds of Tunisia at 60d after flowering (DAF); TS2. Seeds of Tunisia at 120DAF; SS1. Seeds of Sanbai at 60DAF; SS2. Seeds of Sanbai at 120DAF; FC. Fold change; SS1_SS2, TS1_TS2, SS1_TS1 and SS2_TS2. Comparison group, text before ‘_’ represents reference group, text after ‘_’ represents treatment group.

图3　石榴*SUT*基因在不同品种籽粒发育中的表达

Fig. 3　Expression of *SUT* during the development of seed in pomegranates

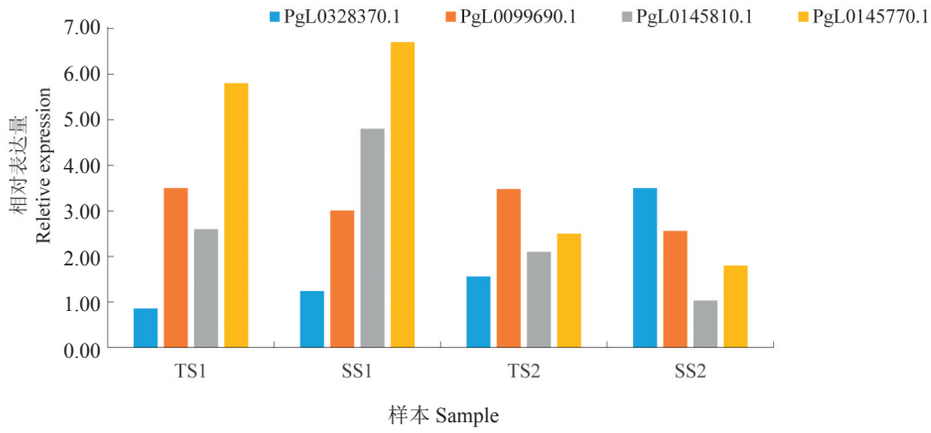


图4 *SUTs*基因在石榴籽粒中的实时荧光定量PCR分析

Fig. 4 qRT-PCR analysis the expression of *SUTs* in the seed of pomegranate

3 讨 论

SUT /*SUC* (Sucrose transporter /carrier) 是 MFS 家族中的一个亚家族 GPH /cation symporter family 的一个分支，在生物进化及重要性状形成过程中扮演重要角色[29-30]。本研究发掘了石榴基因组水平上所有的 *SUT* 基因家族成员，共鉴定了 10 个 *SUT* 基因，其数目与拟南芥的(*AtSUT*1~9)相似。与水稻 *SUT* 基因家族成员(*OsSUT*1~5)相比[31]，石榴 *SUT* 基因家族成员数目较多。因此，进一步研究石榴 SUC 基因家族成员进化及功能，对揭示石榴重要性状分化和形成具有重要作用。

GC 含量是生物核苷酸序列成分组成的标志性指标[32]。不同生物之间 GC 含量存在着较大差异，这种差异某种程度上造成了物种间的分化。目前，中性说和选择说被认为是解释 GC 含量差异的广流学说[33]。中性说认为生物间不同的 GC 含量是由碱基的随机突变和漂移造成的，选择说则认为 GC 含量的差异是由生物不同的生活环境及生活习性等因素共同选择造成的。迄今，大量研究表明真核生物基因组 GC 含量与生活环境具有显著相关性[34]。根据基因 GC 含量的不同，植物基因可划分为高 GC 含量和低 GC 含量两种类型[35]，并且 GC 含量越高，核酸分子结构越稳定。‘突尼斯’石榴基因组 GC 含量 40.38%，介于拟南芥(36%)和水稻基因组(44%)GC 平均含量之间[36-37]。在本研究中，石榴 *SUT* 家族基因成员的平均 GC 含量为 44.35%，低于拟南芥和水稻 *SUT* 家族基因的 GC 平均含量[31]，可能与石榴 *SUT* 基因的平均长度高于拟南芥(1 538.7 bp)和水稻(1 608.6 bp)相关。另外，石榴 *SUT* 家族基因成员的 GC 含量远高于启动子序列的 GC 含量(38.79%)，该研究结果与拟南芥和水稻相似[31]。一方面，*SUT* 基因编码保守性较高的功能蛋白，较高的 GC 含量对维持基因自身的稳定性具有显著作用；另一方面，该结果也与启动子区序列在进化过程中随着生物生长环境的变化更易发生变异的事实相吻合。

启动子是 DNA 上一段能起始基因转录的序列，分为组成型启动子、诱导型启动子和组织器官特异型启动子。组成型启动子如 CaMV35S 启动子、Ubiquitin 启动子等能使外源基因在植物体整个生命周期及所有器官均表达，诱导型启动子能使外源基因在特定诱导条件下表达，组织特异型启动子能使外源基因在植株特定部位表达，在植物生长发育中起着重要作用[38]。Singer 等[39]发现脐橙蔗糖合成酶 1 (*CsSUS1p*) 基因的启动子可以驱动外源基因在维管束韧皮部中表达，可应用于防治维管束疾病。本研究证实石榴 *SUT* 基因成员的启动子区含有 10 种 MYB 元件。这些 MYB 元件与植株生长发育、果实着色、逆境响应相关，暗示 *SUT* 基因可能参与多种生物学过程。

高通量测序技术以及生物信息学分析技术的快速发展，使得转录组学被广泛的应用于生物复杂数量性状遗传机理研究[40-41]。骆翔等[42]采用转录组学手段鉴定了“三白”石榴品种籽粒发育相关差异表达基因，这些差异表达基因显著富集在激素信号转导及其它代谢产物的合成和代谢通路上。Xue 等[4]和 Luo 等[7]采用比较转录组学的方法证实

NAC1, WRKY, MYB 和 MYC 等转录因子参与了石榴籽粒硬度形成。本研究首次揭示了 *SUC* 基因参与调控石榴籽粒发育。对于同一品种来说，*PgL0099690.1*、*PgL0145810.1* 和 *PgL0145770.1* 的表达量随着籽粒的发育逐渐降低，而 *PgL0328370.1* 的表达量逐渐升高，推测这些基因可能与籽粒的发育和增大相关。对于同一时期不同品种来说，*PgL0145770.1* 在硬籽‘三白’品种中的表达量要显著高于软籽‘突尼斯’，表明该基因可能是决定籽粒硬度的关键因子。总之，这些 *SUT* 基因为我们进一步研究石榴籽粒发育提供了关键候选基因。

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