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石榴籽粒硬度研究进展

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摘 要:石榴果实营养丰富、风味独特,具有良好的营养保健功能。随着人们对石榴营养保健功能认识的进一步加深,石榴的市场需求逐渐增大,产业得到了迅猛发展。籽粒硬度作为鲜食石榴的重要品质指标,直接影响着果实的感官品质和消费者的认可度。籽粒硬度已成为近年来石榴研究的热点。从石榴籽粒硬度的形成、石榴籽粒硬度的评价、影响石榴籽粒硬度的因素、石榴籽粒硬度的遗传基础研究等方面进行了全面综述,并介绍了我国自主选育的软籽及半软籽石榴新品种。结合已有研究基础,提出了研究中存在的不足及今后的研究展望。旨在为进一步开展石榴籽粒硬度形成机制与调控技术研究提供方向,为进一步开展石榴籽粒硬度的遗传改良及新品种选育提供指导。

关键词:石榴;籽粒硬度;硬度形成;评价;影响因素;遗传基础

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Progresses in research on pomegranate seed hardness

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Abstract: Pomegranate (*Punica granatum* L.) is a fruit species cultivated worldwide. Pomegranate fruits possess rich nutrition, unique flavor and important economic value, and it is favorite by people for its nutrients benefiting to human health. As more attentions are being paid to the nutrition and health protection functions of pomegranate fruits, its market demand has increased rapidly and the industry has developed gradually. Pomegranate cultivars can be divided into soft-seeded cultivars, semi-soft-seeded cultivars, hard-seeded cultivars, and so on. Seed hardness is one of the important quality characteristics of fresh pomegranate, which determines the sensory quality of the fruit and consumer acceptance. In recent years, with the increasing attentions on the seed hardness of pomegranate fruits, there were many studies focusing on the seed hardness formation and soft-seed pomegranate breeding, so as to understand the reason for pomegranate seed hardness, and provide a reference to further study the genetic mechanism of seed hardness formation in pomegranate. In the present review, seed hardness formation and its influencing factors are reviewed, and candidate genes involving in seed hardness formation and the heredity patterns of seed hardness are summarized. As we known, there are two layers of seed coats for pomegranate, that is, outer and inner seed coats. The soft and juicy outer seed coat is rich in nutrients, which is the main edible part of pomegranate fruits. The inner seed coat is rich in lignin and cellulose, which forms the seed hardness. The seed hardness formation is a process of lignin accumulation in endoderm cells and thickening of secondary cell walls, namely, a process of lignification of endoderm cell walls. The seed hardness is depended on cultivars, growing technology and environment, while the genetics of cultivars plays the decisive roles. Lignin is composed of the primary monolignols

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p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, which form predominantly by oxidative polymerization of the three major monolignols to generate the hydroxyphenyl (H), guaiacyl (G), and syringyl (S) lignin subunits. Nontarget metabolic profiling revealed that coniferyl alcohol and sinapyl alcohol were main monolignols accumulated in seed coat of pomegranate, while little *p*-coumaryl alcohol was detected in the inner seed coat. Thus, S lignin and G lignin appeared to be the dominant lignin subunits in pomegranate seed coats. In addition, the accumulation of cellulose and hemicellulose in seed coat was significantly different between hard-seed and soft-seed pomegranates, which suggested that the accumulation difference of cellulose and hemicellulose in seed coat was also an important factor in the formation of pomegranate seed hardness. Several genes involving in lignin, cellulose and hemicellulose synthesis and degradation had special expression during seed hardness formation, which were identified and considered as the potential candidate genes involving in seed hardness formation. *Pgr011171.1* encoding POD, *Pgr013634.1* encoding F5H, *Pgr022328.1*, *Pgr006310.1*, *Pgr006318.1*, and *Pgr006319.1* encoding CAD, and *Pgr011171.1* encoding POD had lots of transcript accumulation, accompanied with the sinapyl alcohol and coniferyl alcohol accumulation, were candidate genes involving in seed hardness formation. Basing on the results of co-expression network analysis of lignin, cellulose and hemicellulose metabolism-related genes and the transcription factors for inner seed coats at different developmental stages with both hard-seed cultivar and soft-seed cultivar, *Pgr000815.1* encoding AUX-IAA might be involved in the lignin metabolism, and *Pgr011491.1* and *Pgr022940.1* encoding AUX-IAA2, and *Pgr017424.1* encoding NAC66 might be involved in the metabolism of cellulose and hemicellulose. *PgL0137670* encoding NAC transcript factor has a SNP site (T−C) at 166 bp, and the allelic variant T site of *PgL0137670* is associated with the characteristics of soft-seed, according to the comparative transcriptome analysis of soft-seeded cultivar and hard-seeded cultivar. Besides, a number of selective sites related to seed hardness including *SUC8*, *SUC6*, *FOXO* and *MAPK* genes were selected basing on the whole genome resequencing analysis with different seed hardness varieties. However, the gene functions need further studies. The growth environment also has a certain impact on the seed hardness of pomegranate. Analysis of the seed hardness of pomegranate grown in different soils found that the seed hardness of pomegranate grown in sandy soil was the highest, followed by loam soil and the smallest in red loam soil. The study on the effect of different fertilization treatments on the seed hardness of pomegranate found that the application of phosphorus fertilizer could reduce the seed hardness, while the application of fertilizer such as urea, compound fertilizer and potash fertilizer had no significant effect on the seed hardness. Gibberellin, 2,4-dichlorophenoxyacetic acid (2,4−D) and naphthalene acetic acid are widely used plant growth regulators, and they have significant effects on improving fruit quality. Research showed that foliar spraying of these growth regulators during pomegranate flowering could significantly reduce the seed hardness of pomegranate. Although the study of hormones in regulating the seed hardness of pomegranate has been reported in related studies, in the actual production process, there is still a lack of suitable plant growth regulator application technology to promote pomegranate seed softening. In conclusion, seed hardness is one of the characteristics of pomegranate. Although different cultivation environments or artificial measures have a certain impact on the seed hardness of pomegranate, this effect is relatively limited, and genetics is the fundamental factor that determines seed hardness. Finally, the new pomegranate cultivars, bred independently in China and having soft or semi-soft seeds, are introduced in the review. Summarily, the review will provide not only the insight to further study on mechanism and regulation of seed hardness formation, but also the guidance for genetic improvement and breeding in pomegranate.

Key words: Pomegranate; Seed hardness; Formation; Evaluation; Influence factors; Genetic basis

石榴（*Punica granatum* L.）是桃金娘目千屈菜科（Lythraceae）石榴属植物，是最早被人类认识到可以食用的果实之一。据不完全统计，我国现有石榴栽培面积约为15万hm^2[1]，是世界重要的石榴生产国。石榴果实风味独特，营养丰富，并具有抗炎、抗氧化、抗菌等功能而被广泛利用。对鲜食石榴来说，籽粒硬度直接影响着果实的口感和消费者认可度，是石榴果实重要的品质指标。软籽石榴出汁率高、食用方便，而我国现有软籽石榴的品种资源极少，引进的突尼斯软籽等软籽品种由于抗寒性差、坐果不稳定等原因而使栽培面积受限。软籽作为石榴果实重要的性状，已引起育种者的广泛关注，关于籽粒硬度形成机制和遗传规律研究已成为近年的研究热点。笔者将从石榴籽粒硬度的形成与评价、影响籽粒硬度的因素、籽粒硬度相关基因的筛选与挖掘、我国软籽石榴新品种的选育等方面进行一个系统总结，并对未来研究方向进行展望，以期为进一步认识石榴籽粒硬度的形成机制、开展石榴籽粒硬度的遗传改良及新品种选育提供指导。

1 石榴籽粒硬度的形成与评价

1.1 石榴种皮发育与籽粒硬度的形成

石榴籽粒（即种子）由种皮、胚乳和胚三部分组成，种皮又分为外种皮和内种皮，外种皮柔软多汁、营养丰富，是石榴果实的主要食用部位，内种皮质地致密，富含木质素、纤维素，是构成石榴籽粒硬度的基础。对石榴种皮的显微结构观察发现，外种皮由薄壁细胞层和栅栏组织2层细胞组成，内种皮由木栓层、厚壁细胞层以及薄壁细胞层3层细胞组成[2]。从植物发育角度看，种皮由珠被发育而来。石榴和多数被子植物一样具有双珠被，不同的是，拟南芥等植物的内珠被在发育过程中逐渐退化，外种皮积累了更多的原花青素，最后发育成坚硬的种皮；而石榴的两层珠被均正常发育，最后形成了两层种皮，外种皮柔软多汁，内种皮坚硬。从植物进化的角度看，鲜艳、芬芳的花、果可以吸引动物或昆虫进行种子传播和后代繁衍，坚硬的种皮或果皮可以保护胚，避免被动物或昆虫直接消化。可见，石榴鲜艳、多汁的外种皮和质地坚硬的内种皮，都是其长期选择进化的结果。从基因进化的角度看，石榴约在9160万年前从桃金娘目植物中分化形成，外种皮发育标签基因INNER NO OUTER（INO）及其转录调控因子BELL1（BEL1）在基因组进化过程中受到了正向选择[3]，且在外种皮有特异表达；关于内种皮进化的相关基因研究仍需进一步开展。

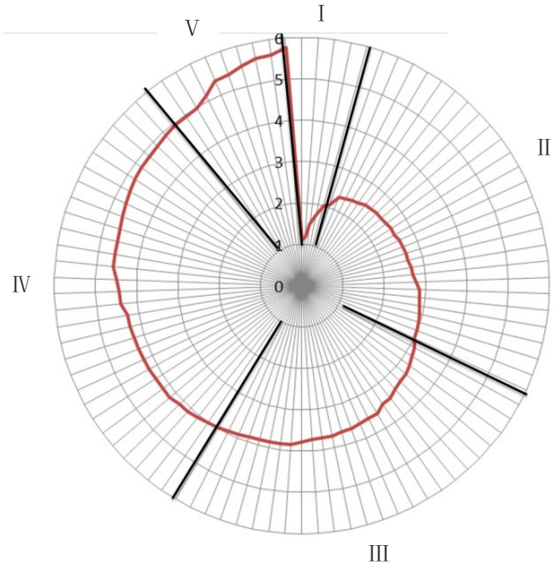
研究发现，石榴种皮在花后40 d开始发育[4]。石榴籽粒硬度的形成是内种皮细胞木质素不断积累、次生细胞壁逐渐加厚的过程，即内种皮细胞壁木质化的过程。作为细胞壁的主要组成成分，木质素与纤维素、半纤维素等相互连接形成网状结构，构成植物细胞的骨架。木质素和纤维素含量分别占石榴种子干物质的21.44%和18.71%[5]。有研究发现，硬籽石榴的种皮细胞壁厚度明显大于软籽石榴[6]。此外，通过解剖观察发现，软籽石榴籽粒内种皮只有部分细胞木质化，然而在硬籽石榴的内种皮中除了维管束周围的细胞，整个内种皮都出现木质化[7]。还有研究发现，籽粒硬度与其木质素含量呈显著正相关[8-9]。自然界中，木质素是由香豆醇（coumaryl alcohol）、松柏醇（coniferyl alcohol）和芥子醇（sinapyl alcohol）3种主要的单体以多种不同的化学键连接而形成。对石榴种皮代谢物的研究发现，石榴内种皮积累的主要木质素单体是芥子醇和松柏醇[10]，说明芥子醇、松柏醇是石榴内种皮木质素积累及籽粒硬度形成的主要物质基础。

1.2 石榴籽粒硬度的评价

关于石榴籽粒硬度的评价方法主要有感官评价[11]、纤维素含量等理化指标测定[11-14]、木质素染色法[11]以及籽粒硬度大小测定等方法。有关籽粒硬度的测定与评价，《石榴籽粒硬度评价方法及影响因素研究》一文已做了较为详细的分析[15]。在质构仪被广泛应用之前，国内对石榴籽粒硬度的测定多采用果实硬度计或谷物硬度计测定[16-17]，但硬度计测定籽粒硬度时，结果稳定性不高；质构仪可以模拟口腔的咀嚼运动，根据果实自身特点选取测试探头并编辑特定运行程序自动分析样品，结果的稳定性和重复性相对较高。近年来广泛开展的用质构仪测定石榴籽粒硬度的研究中，由于选用品种的不同[6,9,13,18-19]，不同研究所测结果的范围有一定差异，在1.7~10 $kg\cdot cm^{-2}$间均有分布。

在籽粒硬度研究的基础上，有学者将石榴分为软籽石榴（种子可食）、半硬籽石榴和硬籽石榴（种子不可食）[11]；有学者将石榴分为软籽、半软籽和硬籽三类[12]，即籽粒硬度＜4 $kg\cdot cm^{-2}$的为软籽石榴，籽粒硬度在4~10 $kg\cdot cm^{-2}$之间的为半软籽品种，籽粒硬

度＞10 kg·cm^{-2}的为硬籽石榴；也有学者认为籽粒硬度＜3.67 kg·cm^{-2}为软籽品种，籽粒硬度在3.67~4.20 kg·cm^{-2}之间的为半软籽品种，籽粒硬度＞ 4.2 kg·cm^{-2}的为硬籽品种；还有学者认为石榴籽粒硬度应该划分为更细的等级，如硬籽、半硬籽、半软籽、软籽、超级软籽等。笔者对148份中国石榴资源的籽粒硬度分布情况（图1）进行研究，发现石榴籽粒硬度呈一个连续的变化，对等级的划分没有也不可能有严格的界限。今后的研究可以以广泛栽培和认可的软籽、硬籽及半软籽石榴为参照，对籽粒硬度划分一个合理的范围。



1~6 代表籽粒硬度大小，Ⅰ~Ⅳ代表籽粒硬度区间。

1-6 are values of seed hardness, Ⅰ-Ⅳ are ranges of seed hardness.

图1　种质水平上石榴籽粒硬度的分布情况（×10^2 N·cm^{-2}）

Fig. 1　Distribution of seed hardness of pomegranate germplasm resources (×10^2 N·cm^{-2})

不仅自然界存在籽粒硬度的多样性，籽粒硬度也有丰富的遗传多态性。由于石榴籽粒由不同心皮发育而来，每个籽粒都代表不同的个体。同一果实内籽粒硬度分布的离散性（图2）进一步说明了籽粒硬度的遗传多态性[11]。由于同一果实内不同籽粒硬度存在着多样性，因此对石榴籽粒硬度评价时测定样本只有达到一定的统计学数量，才能反映果实的籽粒硬度。

2　影响石榴籽粒硬度的因素

遗传、环境因素对籽粒硬度均有一定影响。花粉直感现象，即花粉对受精形成的种子和果实性状

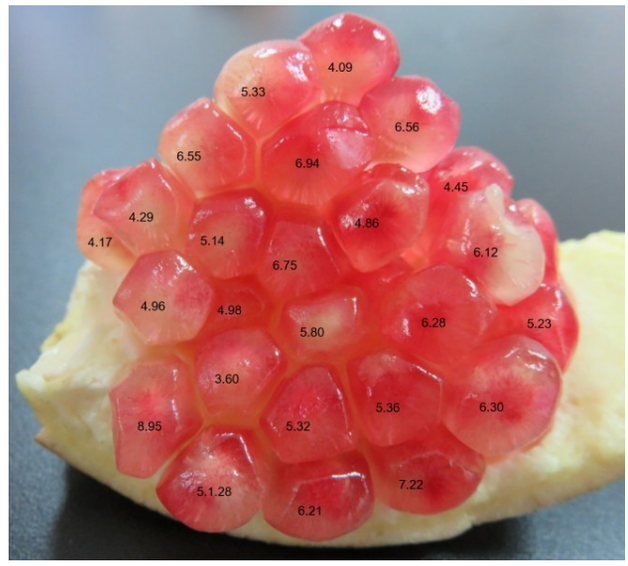


图2　大笨子石榴果实内籽粒硬度的分布（×10^2 N·cm^{-2} [9]）

Fig. 2　The hardness of seeds at different loci in the fruit of Dabeizi (×10^2 N·cm^{-2} [9])

的影响，在果树中普遍存在[20]，石榴中，也存在花粉直感现象[21-22]。在以突尼斯软籽石榴为母本的杂交后代中，籽粒硬度均因父本不同而有差异[7]，自交后代中果实的籽粒硬度最小，说明花粉对石榴籽粒硬度会产生直感效应。对不同树龄突尼斯软籽的籽粒硬度分析发现，籽粒硬度与树龄有明显的相关性，即随着树龄增加，其籽粒硬度有一定下降[23]。对不同着生部位石榴果实的籽粒硬度研究发现，树冠北面和内膛果实的籽粒较软，南面和西南面的籽粒较硬，东面居中[16]。这种差异可能与不同着生部位果实的营养条件和生长微环境不同有关。

对不同土壤中栽培的突尼斯软籽石榴的籽粒硬度分析发现，砂黏土中籽粒硬度最大，壤土次之，红壤土中最小[24]。不同施肥处理对石榴籽粒硬度影响的研究发现，增施磷肥可以降低石榴籽粒的硬度，尿素、复合肥、钾肥等肥料与种子硬度无显著相关性[24]。花蕾期，喷施硼砂（B）300 mg·L^{-1}+KH_2PO_4 300 mg·L^{-1}+尿素（N）500 mg·L^{-1}，不但显著促进石榴坐果、提高产量，也可以使突尼斯石榴籽粒变软[25]。赤霉素、2,4-二氯苯氧乙酸（2,4-D）、萘乙酸是果树栽培中广泛使用的植物生长调节剂。有研究发现，石榴头花始花期至盛花期叶面喷施10.0 mg·L^{-1} GA_3可使突尼斯软籽石榴的籽粒硬度变软，而用GA_3+2,4-D涂抹石榴花柄可显著增加石榴籽粒硬度[26]；初花期和盛花期综合使用75%赤霉酸、0.1%氯吡脲2种植物生长调节剂，也可促使石榴籽软化[27]。尽管植物

生长调节剂在调控石榴籽粒硬度中已有相关研究，但由于对植物生长调剂的种类、浓度及使用方法等缺乏系统研究，目前尚无成熟可用的生产技术。笔者对10个产区13个产地突尼斯软籽石榴的籽粒硬度研究发现，不同产区不同产地突尼斯软籽石榴的籽粒硬度变化较小[28]。

综上所述，适当的人工措施对石榴籽粒硬度有一定影响，但这种影响比较有限，并不能稳定、长期地改变籽粒的硬度。籽粒硬度作为石榴品种的特征之一，是一个相对稳定的性状，主要受品种本身的遗传特性决定。

3 石榴籽粒硬度的遗传基础

作为石榴内种皮木质化的重要物质基础，多酚聚合物木质素、多糖聚合物纤维素和半纤维素的合成、代谢与积累是研究石榴籽粒硬度形成机制的关键。木质素是苯丙氨酸或酪氨酸在一系列酶的催化下形成木质素单体，然后在聚合酶的作用下最终聚合而成[29]。木质素单体的形成与聚合在模式植物中已比较清楚[30-31]。在全基因组密码破译之前，通过转录组分析和同源克隆，从石榴籽粒中获得了一些参与木质素合成与聚合的基因和转录调控因子[13,32-35]。根据对石榴软籽性状基因连锁标记的研究，推测石榴籽粒硬度由微效多基因控制[36]。石榴全基因组遗传密码的破译，为全面解析石榴重要性状的遗传基础奠定了重要基础。Qin等[3]首次在全基因组信息水平上注释出了石榴木质素、纤维素、半纤维素代谢相关基因，并通过对软硬籽石榴不同发育时期内外种皮的基因表达谱研究筛选出了部分在硬籽品种及籽粒硬度形成关键时期特异表达的基因，推测籽粒硬度形成与木质素、纤维素、半纤维素的合成及降解相关基因的特异表达有关。该推论在软硬籽石榴的蛋白组研究中得到了进一步验证[37]。由于参与木质素、纤维素及半纤维素合成和降解的相关基因主要为多基因家族，因此进一步聚焦候选基因变得十分必要。Luo等[38]在以不同籽粒硬度品种为材料进行全基因组重测序分析研究中，选出了包含SUC8、SUC6、FOXO和MAPK基因在内的多个籽粒硬度相关选择性位点，但由于选择群体相对较小，筛选到的选择位点比较有限，且具体的功能还有待进一步的验证。针对特定代谢物的多组学联合分析筛选候选基因，具有筛选效率高、针对性强的特点而被广泛采用[39]。对软硬籽石榴不同发育时期的内外种皮的基因组、转录组及代谢组数据进行联合分析，根据正向法则，筛选出了与硬籽石榴中木质素单体芥子醇大量积累相关的候选基因POD（*Pgr011171.1*）及与木质素单体松柏醇积累相关的候选基因F5H（*Pgr013634.1*）、CAD（*Pgr022328.1*，*Pgr006310.1*，*Pgr006318.1*，and *Pgr006319.1*）和POD（*Pgr011171.1*）[10]。对17个纤维素和半纤维素代谢关键酶基因的表达谱研究发现，有一些基因在果实发育过程中随着籽粒硬度的增加而有特异性表达。其中，被证明以复合体参与植物次生壁形成的CesA 4, CesA 7和CesA 8[40]，其编码基因*Pgr007560*、*Pgr012627*和*Pgr027371*基因在石榴内种皮发育过程中有特异性高表达[10]，因此，也可能参与石榴籽粒硬度的形成。此外，对不同籽粒硬度石榴内外种皮发育过程中木质素、纤维素、半纤维素代谢相关基因和相关转录因子的共表达网络分析，筛选出AUX-IAA（*Pgr000815*）可能参与石榴种皮木质素代谢，而AUX-IAA2（*Pgr011491.1*）、MYB（*Pgr022940.1*）和NAC66（*Pgr017424.1*）可能参与纤维素和半纤维素的代谢[10]。对不同籽粒硬度内种皮MicroRNA的研究发现，参与调控木质素合成的转录因子MYB、NAC和WRKY可能受miR164e和miR172b的调控[41]，通过异源转化证明了转录因子*PgSND1-like*参与了木质素的生物合成[42]。*PgL0145810.1*编码的SUT蔗糖转运蛋白可能在石榴籽粒硬度形成过程中起负调控作用[43]。总之，对石榴籽粒硬度遗传基础的研究虽已有一定进展，但相关基因的功能研究尚在起步阶段，相关的代谢及调控网络研究也有待进一步的深化。

分子标记辅助育种已成为性状定向改良的有效手段，籽粒硬度相关分子标记研究也已成为关注和研究的重点。陆丽娟[36]在2006年成功的将一个RAPD标记转化为SCAR，该标记可成功辨别软籽石榴白玉石籽和硬籽石榴三白。Harel-Beja等[44]利用关联分析在全基因中发现4个籽粒硬度关联的QTL位点，分别解释表型变异15%~30%。通过比较突尼斯和三白转录组，发现NAC（*PgL0137670*）在166 bp处存在一个SNP位点（T-C），并发现*PgL0137670*的等位变异T位点和软籽性状关联[42]。但是由于这些标记都是在特定品种中筛选的，通用性并不强，随着测序技术的发展，硬、软籽石榴的基因组都已

成功破译，通过对软籽和硬籽石榴的比较基因组研究发现，在1号染色体上发现一段26.2 Mb的受选择区域，推测其可能是石榴硬籽和软籽特性分化的区段[38]，今后，可在该区段内开发出软籽性状关联的分子标记，并应用于软籽石榴的分子标记辅助育种。

4　我国软籽石榴新品种的选育

我国传统石榴资源中，主要是硬籽和半软籽石榴，现有调查和研究中未发现原产我国的软籽品种。以突尼斯软籽石榴为代表的软籽石榴品种因食用方便而倍受消费者青睐，在我国开始大面积发展。关于软籽石榴的新品种选育工作也不断展开，近年来我国自主培育的软籽石榴品种不断涌现[45-58]（表1），为石榴产业的健康快速发展奠定了重要基础。

表1　我国自主培育的软籽石榴新品种

Table 1　New pomegranate cultivars with soft or semi-soft seeds bred independently in China

品种 Cultivar	品种来源及选育方法 Cultivar origin and breeding methods	果实主要性状 Main fruit traits	选育单位 Breeding organization	选育年份 Breeding year
会理青皮软籽[46] Huiliqingpiruanzi	会理石榴三白芽变选种 This is a bud mutation of local cultivar Sanbai in Huili country	果大、色鲜、皮薄、粒大、汁多、核软、香甜（带有蜂蜜味）、味浓。 It has a big fruit, bright-colored peel and thin rind. The seed is big, soft and juicy. Fruit is sweet and has good flavor.	四川省会理县石榴研究所 Institute of Huili Pomegranate Research	1999
淮北软籽1号[47-48] Huaibeiruanzi 1	淮北黄里软籽石榴芽变选优 This is a bud mutation of local cultivar Huaibeihuangliruanzishiliu	果皮光洁较薄，籽粒白色有红色针状晶体，果实种核软，品质上等。 The peel is bright and the rind is thin. Aril is white with red brilliant rays. Seed is soft. Fruit quality is good.	安徽省淮北市林业局 Huaibei Forestry Bureau	2002
淮北软籽2号[47-48] Huaibeiruanzi 2	淮北黄里软籽石榴芽变选优 This is a bud mutation of local cultivar Huaibeihuangliruanzishiliu	果皮光洁、较厚，青绿色，红晕明显，籽粒红色，针状晶体明显，核软，品质上等。 The peel is green with red blush, its surface is bright and clean. Aril is red with brilliant rays. Seed is soft. Fruit quality is good.	安徽省淮北市林业局 Huaibei Forestry Bureau	2002
淮北软籽3号[47-48] Huaibeiruanzi 3	淮北黄里软籽石榴芽变选优 This is a bud mutation of local cultivar Huaibeihuangliruanzishiliu	果皮较薄，青黄色，籽粒白色，可见辐射状晶体，核绵软。 The peel is green-yellow and the rind is thin. Aril is white with brilliant rays. The seed is soft.	安徽省淮北市林业局 Huaibei Forestry Bureau	2002
红玉石籽[49] Hongyushizi	怀远玉石籽营养系变异 Variation of a local variety Huaiyuanyushizi	果皮光滑深红，籽粒淡黄，顶端淡红，汁多籽软，味甜酸，品质上。 The peel is crimson red and its surface is bright. Aril is yellowish with red in the apex. It has sweet-sour taste and soft seed.	安徽农业大学 Anhui Agricultural University	2003
红玛瑙籽[50] Hongmanaozi	玛瑙籽营养系变异 Variation of a local variety Manaozi	果皮底色橙黄，阳面红晕或斑点，味甘甜，籽核软，籽粒较大，品质佳。 The peel is green-yellow with red blush on the sunny side. Seed is big and soft. Fruit is sweet and has good quality.	安徽农业大学 Anhui Agricultural University	2003
范村软籽[51] Fancunruanzi	河南开封地方名优品种 Famous local variety in Kaifeng, Henan Province	果皮鲜红，籽粒红玛瑙色，味浓甜，成熟度越高籽粒越软，品质上佳。 The peel is bright red and arils are agate. Fruit tastes sweet. Seed becomes soft with fruit development.	河南开封农业科学研究所 Institute of Kaifeng Agricultural Sciences	2007
红如意[45] Hongruyi	由突尼斯软籽×粉红甜杂交后代 Hybrid offspring of Tunisiruanzi and Fenpitian	平均单果质量410.0 g; 百粒重56.0 g; 果皮鲜红色；可溶性固形物含量15.1%；北方产区9月上旬成熟。 Average fruit mass is more than 410.0 g. 100-seed weight is 56.0 g. The peel is bright red. Soluble solid content is 15.1%. The fruit ripens in early September.	河南省农业科学院 Henan Academy of Agricultural Sciences	2007
白玉石籽[52] Baiyushizi	怀远三白石榴营养系变异 Variation of a local variety Sanbai	花、果皮和籽粒均呈黄白至乳白色，果型大，籽粒大，味甜，核较软。 The flower, peel and aril are light yellow to yellowish-white. It has large fruit and big seeds. Seeds are semi-soft and taste sweet.	安徽农业大学 Anhui Agricultural University	2009

表1 （续）

Table 1 （continued）

品种 Cultivar	品种来源及选育方法 Cultivar origin and breeding methods	果实主要性状 Main fruit traits	选育单位 Breeding organization	选育年份 Breeding year
枣选1号[53] Zaoxuan 1	郭村软籽石榴营养系变异 Variation of a local variety Guocunruanzi	果面光洁鲜红，籽粒青白透明，核软可食。 The peel is red and surface is bright and clean. Arils are white and seeds are soft.	枣庄市市中区林业局 Zaozhuang Shizhong District Forestry Bureau	2007
枣选3号[53] Zaoxuan 3	郭村软籽石榴营养系变异 Variation of a local variety Guocunruanzi	果面光洁鲜红，籽粒略红透明，核软。 The peel is bright red, its surface is bright and clean. The arils are light red and the seeds are soft.	枣庄市市中区林业局 Zaozhuang Shizhong District Forestry Bureau	2007
中农红软籽石榴[54] Zhongnonghong ruanzishiliu	突尼斯软籽石榴的营养系变异 Variation of Tunisiruanzi	果实近圆球形，果皮光洁明亮，阳面浓红色，平均单果质量475 g，籽粒紫红色,汁多味甘甜，核特软，风味极佳。 The fruit is nearly round. Its surface is bright. The peel is crimson in sunny side. The average fruit mass is 475 g. The arils are crimson red in color. The fruit is juicy, sweet, soft-seeded and having good flavor.	中国农业科学院郑州果树研究所 Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences	2010
红双喜[45] Hongshuangxi	突尼斯软籽×豫大籽杂交选育 Hybrid offspring of Tunisiruanzi and Yudazi	果皮鲜红色，平均单果质量410.0 g，百粒重56. 0 g，籽粒出汁率91.4%，可溶性固形物含量15.1%，北方产区9月上旬成熟。 The peel is bright red. Average fruit mass is more than 410.0 g. 100-seed weight is 56.0 g. The yield of juice was 91.4%. Soluble solid content is 15.1%. Generally, it ripens in early September.	河南省农业科学院 Henan Academy of Agricultural Sciences	2012
红玉软籽[45] Hongyuruanzi	突尼斯软籽石榴的营养系变异 Variation of Tunisiruanzi	平均单果质量602.0 g，百粒重56.2 g，成熟时果实向阳面红色，背阴面红色占1/2，其余为黄青色。 Average fruit mass is more than 602. 0 g. 100-seed weight is 56. 2 g. Fruit surface is red in sunny side, while the rest is red and yellow-green in half.	攀枝花市农林科学院、凉山州亚热带作物研究所、四川省农科院园艺研究所 Panzhihua Academy of Agricultural and Forestry Science, Liangshan Subtropical Crops Research Institute and Sichuan Academy of Agricultural Sciences	2015
紫美[45] Zimei	以色列引进品种 A cultivar imported from Israel	果大，果皮紫红色，籽粒紫红，核软可食。 It has big fruits. The peel and aril are purplish red, the seed is soft and edible.	攀枝花市农林科学院、凉山州亚热带作物研究所、四川省农科院园艺研究所 Panzhihua Academy of Agricultural and Forestry Science, Liangshan Subtropical Crops Research Institute and Sichuan Academy of Agricultural Sciences	2015
中石榴2号[55] Zhongshiliu 2	突尼斯软籽×豫大籽杂交选育 Hybrid offspring of Tunisiruanzi and Yudazi	果皮光洁明亮，果面红色，着色率在85%以上，籽粒红色，汁多味酸甜，出汁率85.7%，核仁半软，风味甘甜可口，品质极佳。 The fruit surface is bright and clean. 85% of the surface is red. Arils are red. Fruit is juicy and sour-sweet. The seeds are hemi-soft and taste well.	中国农业科学院郑州果树研究所 Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences	2016
慕乐[56] Mule	以色列引进品种 A cultivar imported from Israel	果皮光洁明亮，粉红色，籽粒红色，汁多味甜，核软，品质极佳。 The fruit is bright and clean. Its peel is pink, while seed is red. It is juicy, soft-seeded, sweet, and taste well.	河南林业职业学院、中国农业科学院郑州果树研究所 Henan College of Forestry, Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences	2018
玛丽斯[57] Malisi	以色列引进品种 A cultivar imported from Israel	果皮光洁红色，籽粒红色，汁多味甜，核仁极软。 The fruit is bright and clean. The peel and aril is red. The seed is completely soft. It is juicy and taste sweet.	河南林业职业学院、中国农业科学院郑州果树研究所 Henan College Of Forestry, Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences	2018
中石榴8号[58] Zhongshiliu 8	突尼斯软籽×中石榴1号杂交后代 Hybrid offspring of Tunisiruanzi and Zhongshiliu1	果面红色，光洁明亮，籽粒红色，汁多味甜酸，籽粒超软。 The fruit surface is bright and clean. The peel and aril are red. The fruit is sweet-sour and seeds are completely soft.	中国农业科学院郑州果树研究所 Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences	2018

5 存在问题与研究展望

籽粒硬度作为石榴果实的重要品质指标之一，已引起人们的广泛关注。关于籽粒硬度形成的物质基础已基本明确，相关的遗传基础也已展开研究，这为阐明石榴籽粒硬度的形成机制及调控技术研究奠定了重要基础。由于构成籽粒硬度的物质基础极其复杂，涉及的代谢通路及影响因素较多，遗传机制也十分复杂，相关遗传转化体系尚未建立，为基因功能的验证带来了一定困难。在软籽石榴育种方面，国内主栽软籽石榴品种类型单一，抗寒性较差，主栽品种突尼斯软籽在栽培过程中存在籽粒黑化等问题，我国已选育出了多个软籽或半软籽品种，选育方法主要是芽变选种和引种驯化，性状虽有一定改善，但并无突破性进展；杂交育种虽已展开，但由于对软籽性状的遗传规律知之甚少，选育效率相对较低，选育品种也较少。

为加快石榴软籽性状形成机制的研究进程，培育更多综合性状优良的软籽石榴新品种，今后可从以下几个方面开展工作。

（1）针对石榴软籽性状开展遗传规律的研究，通过分离群体定位、群体重测序技术，结合精细表型数据解析软籽性状的遗传位点，发掘关键通用性分子标记，分离关键基因；在此基础上，利用分子标记，以“籽软、综合性状优良、熟期配套”为育种目标，选育满足不同栽培区以及市场需求的新软籽品种，加快软籽石榴的分子标记辅助选育进程。

（2）利用基因组学、转录组学、代谢组学等多组学联合技术，解析石榴籽粒硬度形成的分子机制，对已分离的关键基因开展基因功能研究，阐明重要基因的生物学功能及作用机制，明确其分子调控网络。

（3）明晰影响石榴籽粒硬度形成的因素，并在此基础上开展调控技术的研究，如最佳授粉品种的筛选、最适植物生长植物调节剂的施用等技术。合理应用调控技术，促进高品质、高产的软籽石榴生产。

（4）针对国内主栽软籽石榴品种存在抗逆性差、不耐储存等问题，一方面要加强石榴抗性机制、储藏保鲜技术的理论研究，另一方面要加强石榴种质资源的收集及利用，丰富杂交育种亲本，以期培育出抗性强、籽粒软、风味佳的石榴新品种。

参考文献 References：

[1] 曹尚银，侯乐峰 . 中国果树志 . 石榴卷[M]. 北京：中国林业出版社，2013：6.
CAO Shangyin, HOU Lefeng. China fruit monograph- Punica granatum[M]. Beijing: China Forestry Publishing House, 2013: 8.

[2] 秦改花，刘春燕，徐义流，李艳玲，齐永杰，潘海发，伊兴凯，高正辉 . 石榴种皮结构及发育过程的显微观察[J]. 热带作物学报，2018，39（3）：489-493.
QIN Gaihua, LIU Chunyan, XU Yiliu, LI Yanling, QI Yongjie, PAN Haifa, YI Xingkai, GAO Zhenghui. Microstructure observation of seed coats and its development of pomegranate (*Punica granatum* L.)[J]. Chinese Journal of Tropical Crops, 2018, 39 (3): 489-493.

[3] QIN G H, XU C Y, MING R, TANG H B, GUYOT R, KRAMER E M, HU Y D, YI X K, QI Y J, XU X Y, GAO Z H, PAN H F, JIAN J B, TIAN Y P, YUE Z, XU Y L. The pomegranate (*Punica granatum* L.) genome and the genomics of punicalagin biosynthesis[J]. The Plant Journal, 2017, 91(6): 1108-1128.

[4] 薛辉，曹尚银，陈利娜，李好先，牛娟，张富红，赵弟广 .‘豫大籽’与‘突尼斯’石榴的种子结构及硬度比较[J]. 果树学报，2016，33（5）：563-569.
XUE Hui, CAO Shangyin, CHEN Lina, LI Haoxian, NIU Juan, ZHANG Fuhong, ZHAO Diguang. Comparison of seed structure and hardness between‘Yudazi’and‘Tunisia’[J]. Journal of Fruit Science, 2016, 33(5): 563-569.

[5] DALIMOV D N, DALIMOVA G N, BHATT M. Chemical composition and lignins of tomato and pomegranate seeds[J]. Chemistry of Natural Compounds, 2003, 39(1): 37-40.

[6] 谢小波，黄云，田胜平，李贵利，曹尚银 . 软籽石榴种子硬度发育与种皮细胞壁显微结构的关系研究[J]. 园艺学报，2017，44（6）：1174-1180.
XIE Xiaobo, HUANG Yun, TIAN Shengping, LI Guili, CAO Shangyin. Relationship of seed hardness development and microstructure of seed coat cell in soft seed pomegranate[J]. Acta Horticulturae Sinica, 2017, 44(6): 1174-1180.

[7] PUJARI K H，RANE D A. Concept of seed hardness in pomegranate - I) anatomical studies in soft and hard seeds of‘Muskat’pomegranate[J]. Acta Horticulturae, 2015, 1098: 97-104.

[8] ZAMANI Z, ZAREI A, FATAHI R. Characterization of progenies derived from pollination of pomegranate cv. Malase-Tourshe-Saveh using fruit traits and RAPD molecular marker[J]. Scientia Horticulturae, 2009, 124(1): 67-73.

[9] 秦改花，徐义流，李艳玲，刘春燕，齐永杰，高正辉，潘海发，伊兴凯，刘长华 . 石榴籽粒硬度特征及其相关生理指标研究[J]. 热带作物学报，2018，39（1）：67-71.
QIN Gaihua, XU Yiliu, LI Yanling, LIU Chunyan, QI Yongjie, GAO Zhenghui, PAN Haifa, YI Xingkai, LIU Changhua. Char-

acteristics of seed hardness and its relative physiological indexes in pomegranate[J]. Chinese Journal of Tropical Crops, 2018, 39(1): 67-71.

[10] QIN G H, LIU C Y, LI J Y, QI Y J, GAO Z H, ZHANG X L, YI X K, PAN H F, MING R, XU Y L. Diversity of metabolite accumulation patterns in inner and outer seed coats of pomegranate: exploring their relationship with genetic mechanisms of seed coat development[J]. Horticulture Research, 2020, 7(1): 10.

[11] MELGAREJO P, SANCHEZ M, HERNANDEZ F, MARTNEZ J J, AMOROS A. Parameters for determining the hardness and pleasantness of pomegranates(*Punica granatum* L.)[J]. Options Méditerranéennes.Série A:Séminaires Méditerranéens, 2000, 42: 225-230.

[12] JALIKOP S H, KUMAR P S. Use of soft, semi-soft and hardseedled types of pomegranate (*Punice granatum*) for improvement of firuit attributes[J]. Indian Jounal of Agricultural Sciences, 1998, 68(2): 87-91.

[13] 张水明，龚凌燕，曹丹琴，张永娟，杨健 . 石榴种皮总木质素含量及 PgCOMT 基因的克隆与表达[J]. 热带亚热带植物学报，2015，23：65-73.

ZHANG Shuiming, GONG Lingyan, CAO Danqin, ZHANG Yongjuan, YANG Jian. Total lignin content in pomegranate seed coat and cloning and expression analysis of PgCOMT gene[J]. Journal of Tropical and Subtropical Botany, 2015, 23:65-73.

[14] 张立华，朱晓梅，徐加喜，吕慧 . 影响石榴籽硬度相关因素的分析[J]. 北方园艺，2017(23)：47-51.

ZHANG Lihua, ZHU Xiaomei, XU Jiaxi, LÜ Hui. Related factors affecting hardness of pomegranate seed[J]. Northern Horticulture, 2017(23): 47-51.

[15] 谭伟，张立华 . 石榴籽粒硬度评价方法及影响因素研究[C]// 曹尚银，李好先 . 中国石榴研究进展(三). 北京：中国林业出版社，2018：205-210.

TAN Wei, ZHANG Lihua. Study on the evaluation methods and influencing factors of seed hardness in *Punica granatum* L. [C]// CAO Shangyin, LI Haoxian. The research progress of pomegranate (III). Beijing: China Forestry Publishing House, 2018: 205-210.

[16] 陆丽娟，巩雪梅，朱立武 . 中国石榴品种资源种子硬度性状研究[J]. 安徽农业大学学报，2006，33(3)：356-359.

LU Lijuan, GONG Xuemei, ZHU Liwu. Study on seed hardness of pomegranate cultivars in China[J]. Journal of Anhui Agricultural University, 2006, 33(3): 356-359.

[17] 汪小飞，周耘峰，赵昌恒，孙龙，伍全根 . 36 个石榴(*Punica granatum* L.)品种的品质测定[J]. 热带作物学报，2010，31(1)：136-140.

WANG Xiaofei, ZHOU Yunfeng, ZHAO Changheng, SUN Long, WU Quangen. Determination of fruit quality of 36 pomegranate cultivars[J]. Chinese Journal of Tropical Crops, 2010, 31 (1): 136-140.

[18] 杨子君，安广池，史亚萍，乔若谨，李纪顺，张永刚，郭凯，杨合同 . 山东枣庄 6 个主栽石榴品种的食用品质比较[J]. 食品科技，2015，40(1)：45-50.

YANG Zijun, AN Guangchi, SHI Yaping, QIAO Ruojin, LI Jishun, ZHANG Yonggang, GUO Kai, YANG Hetong. Eating quality comparison of six pomegranate cultivars in Zaozhuang Shandong[J]. Food Science and Technology, 2015, 40(1): 45-50.

[19] 赵登超，贾明，唐贵敏，梁燕，王小芳，舒秀阁，梁静，亓玉昆，孙蕾，屈良静 . 质构穿刺检测石榴籽粒质地品质[J]. 热带作物学报，2016，37(7)：1419-1423.

ZHAO Dengchao, JIA Ming, TANG Guimin, LIANG Yan, WANG Xiaofang, SHU Xiuge, LIANG Jing, QI Yukun, SUN Lei, QU Liangjing. Establishment of the detecting method on the fruit texture of pomegranate by puncture test[J]. Chinese Journal of Tropical Crops, 2016, 37(7): 1419-1423.

[20] 洪俊彦，黄仁，黄春颖，王建华，徐一帆，李佩佩，胡渊渊，黄坚钦，李岩 . 植物花粉直感的研究进展及展望[J]. 植物生理学报，2020，56(2)：151-162.

HONG Junyan, HUANG Ren, HUANG Chunying, WANG Jianhua, XU Yifan, LI Peipei, HU Yuanyuan, HUANG Jianqin, LI Yan. Research progress and prospects of xenia[J]. Plant Physiology Journal, 2020, 56(2): 151-162.

[21] 王艳，杨荣萍，张旻威，洪明伟，李松开，张赛赛，井锐锐，姜勤，李文祥 .5 个石榴品种杂交 F_1 代分析研究初探[J]. 西部林业科学，2016，45(6)：73-78.

WANG Yan, YANG Rongping, ZHANG Minwei, HONG Mingwei, LI Songkai, ZHANG Saisai, JING Ruirui, JIANG Qin, LI Wenxiang. F_1 generation cross seeding of five *Punica granatum* L. cultivars[J]. Journal of West China Forestry Science, 2016, 45 (6): 73-78.

[22] 薛辉，曹尚银，牛娟，李好先，张富红，赵弟广 . 花粉直感对‘突尼斯’石榴坐果及果实品质的影响[J]. 果树学报，2016，33(2)：196-201.

XUE Hui, CAO Shangyin, NIU Juan, LI Haoxian, ZHANG Fuhong, ZHAO Diguang. Effects of xenia on fruit setting and quality in ‘Tunisia’ pomegranate[J]. Journal of Fruit Science, 2016, 33(2): 196-201.

[23] 薛辉 . 软籽石榴种子硬度的影响因素及转录组分析[D]. 北京：中国农业科学院，2016.

XUE Hui. Factors affecting the seed hardness and the transcriptome analysis of soft- seed pomegranate[D]. Beijing: Chinese Academy of Agricultural Sciences Dissertation, 2016.

[24] 薛辉，曹尚银，刘贝贝，李好先，牛娟，张杰 . 不同土壤类型及肥料对突尼斯软籽石榴种子硬度的影响[J]. 江西农业学报，2017，29(1)：43-46.

XUE Hui, CAO Shangyin, LIU Beibei, LI Haoxian, NIU Juan, ZHANG Jie. Effect of different soil types and fertilizer on seed hardness of Tunisian soft- seed pomegranate[J]. Acta Agriculturae Jiangxi, 2017, 29(1): 43-46.

[25] 司守霞，牛娟，陈利娜，曹尚银 . 不同药剂对石榴可育花比率及产量和品质的影响[J]. 西北林学院学报，2017，32(4)：111-

116.
SI Shouxia, NIU Juan, CHEN Lina, CAO Shangyin. Effect of different reagents on the proportion of fertile flower and fruit yield and quality of pomegranate[J]. Journal of Northwest Forestry University, 2017, 32(4): 111-116.

[26] 陈利娜，牛娟，刘贝贝，敬丹，骆翔，李好先，夏小丛，杨选文，张富红，曹达，王启，曹尚银 . 不同花期喷施植物生长调节剂对石榴坐果及果实品质的影响[J]. 果树学报，2020，37（2）：244-253.
CHEN Lina, NIU Juan, LIU Beibei, JING Dan, LUO Xiang, LI Haoxian, XIA Xiaocong, YANG Xuanwen, ZHANG Fuhong, CAO Da,WANG Qi, CAO Shangyin. Effects of foliar application of plant growth regulators at different flowering stages on fruit setting and quality in pomegranate[J]. Journal of Fruit Science, 2020,37(2):244-253.

[27] 丁在喜 . CN 103250580 A：一种软化石榴硬籽的方法[P].2015-04-22.
DING Zaixi. CN 103250580 A: A method of softening the hard seeds of pomegranate[P]. 2015-04-22.

[28] 刘春燕，秦改花，黎积誉，刘长华，钮得文 .‘突尼斯软籽’石榴果实品质评价及主要评价因子分析[C]//曹尚银，李好先 . 中国石榴研究进展（三）. 北京：中国林业出版社，2018：218-224.
LIU Chunyan, QIN Gaihua, LI Jiyu, LIU Changhua, NIU Dewen. Evaluation of fruit quality for ‘Tunisiruanzi’ pomegranate and the analysis of evaluation factors[C]// CAO Shangyin, LI Haoxian. The research progress of pomegranate (III). Beijing: China Forestry Publishing House, 2018: 218-224.

[29] 李潞滨，刘蕾，何聪芬，董银卯，彭镇华 . 木质素生物合成关键酶基因的研究进展[J]. 分子植物育种, 2007，5(S1): 45-51.
LI Lubin, LIU Lei, HE Congfen, DONG Yinmao, PENG Zhenhua. Research progresses on the genes encoding the key enzymes in biosynthetic pathway of lignin[J]. Molecular Plant Breeding, 2007, 5(S1): 45-51.

[30] HUMPHREYS J M, CHAPPLE C. Rewriting the lignin roadmap[J]. Current Opinion in Plant Biology, 2002, 5(3): 224-229.

[31] BOERJAN W, RALPH J, BAUCHER M. Lignin biosynthesis[J]. Annual Review of Plant Biology, 2003, 54(1): 519-546.

[32] 董丽丽，龚凌燕，陈磊，涂佳丽，张水明 . 石榴肉桂酰辅酶 A 还原酶基因的克隆与表达分析[J]. 南京农业大学学报，2016，39（5）：747-753.
DONG Lili, GONG Lingyan, CHEN Lei, TU Jiali, ZHANG Shuiming. Cloning and expression analysis of PgCCR from pomegranate[J]. Journal of Nanjing Agricultural University, 2016, 39(5): 747-753.

[33] 黄蓉，熊枫，陈磊，张水明，董丽丽 . 石榴木质素合成相关基因 PgMYB308 的克隆和表达分析[J]. 西北植物学报，2017，37（12）：2357-2362.
HUANG Rong, XIONG Feng, CHEN Lei, ZHANG Shuiming, DONG Lili. Cloning and expression analysis of lignin biosynthesis-related gene PgMYB308 in pomegranate[J]. Acta Botanica Boreali-Occidentalia Sinica, 2017, 37(12): 2357-2362.

[34] DONG L L, XIONG F, LIU N, WANG Q, ZHANG S M. Molecular cloning and expression analysis of PgLAC in pomegranate[J]. American Journal of Molecular Biology, 2018, 8(3): 145-155.

[35] XUE H, CAO S Y, LI H X, ZHANG J, NIU J, CHEN L N, ZHANG F H, ZHAO D G. De novo transcriptome assembly and quantification reveal differentially expressed genes between soft-seed and hard-seed pomegranate (*Punica granatum* L.)[J]. PloS One, 2017, 12(6): e0178809.

[36] 陆丽娟 . 石榴软籽性状基因连锁标记的克隆与测序[D]. 合肥：安徽农业大学，2006.
LU Lijuan. Cloning and sequence measuring of the marker linked to seed trait in soft-seeded pomegranate[D]. Hefei: Anhui Agricultural University, 2006.

[37] NIU J, CAO D, LI H X, XUE H H, CHEN L N, LIU B B, CAO S Y. Quantitative proteomics of pomegranate varieties with contrasting seed hardness during seed development stages[J]. Tree Genet Genomes, 2018, 14(1): 14.

[38] LUO X, LI H X, WU Z K, YAO W, ZHAO P, CAO D, YU H Y, LI K D, POUDEL K, ZHAO D G, ZHANG F H, XIA X C, CHEN L N, WANG Q, JING D, CAO S Y. The pomegranate (*Punica granatum* L.) draft genome dissects genetic divergence between soft- and hard-seeded cultivars[J]. Plant Biotechnology Journal, 2020, 18(4): 955-968.

[39] 许秋健，李丽，王松标，马小卫，武红霞，许文天，梁清志，陈杰忠 . 代谢组和转录组联合分析果树生理机制研究进展[J]. 果树学报，2020，37（9）：145-156.
XU Qiujian, LI Li, WANG Songbiao, MA Xiaowei, WU Hongxia, XU Wentian, LIANG Qingzhi, CHEN Jiezhong. Review on integrated analysis of the metabolome and transcriptome of physiological mechanism of fruit trees[J]. Journal of Fruit Science, 2020,37(9):145-156.

[40] TAYLOR N G, HOWELLS R M, HUTTLY A K ,VICKERS K, TURNER S R. Interactions among three distinct CesA proteins essential for cellulose synthesis[J]. Proceedings of the National Academy of Sciences of the United States of America, 2003, 100 (3): 1450-1455.

[41] LUO X, CAO D, ZHANG J F, CHEN L, XIA X C, LI H X, ZHAO D G, ZHANG F H, XUE H, CHEN L N, LI Y Z, CAO S Y. Integrated microRNA and mRNA expression profiling reveals a complex network regulating pomegranate (*Punica granatum* L.) seed hardness[J]. Scientific Reports, 2018, 8(1): 9292.

[42] XIA X C, LI H X, CAO D, LUO X, YANG X W, CHEN L N, LIU B B, WANG Q, JING D, CAO S Y. Characterization of a NAC transcription factor involved in the regulation of pomegranate seed hardness (*Punica granatum* L.)[J]. Plant Physiology and Biochemistry, 2019, 139: 379-388.

[43] POUDEL K, LUO X, CHEN L N, JING D, XIA X C, TANG L Y, LI H X , CAO S Y. Identification of the SUT gene family in pomegranate (*Punica granatum* L.) and functional analysis of

PgL0145810.1[J]. International Journal of Molecular Sciences, 2020, 21(18): 6608.

[44] HAREL-BEJA R, SHERMAN A, RUBINSTEIN M, ESHED R, BAR-YA'AKOV I, TRAININ T, OPHIR R, HOLLAND D. A novel genetic map of pomegranate based on transcript markers enriched with QTLs for fruit quality traits[J]. Tree Genetics & Genomes, 2015, 11: 109.

[45] 罗华，刘娜，郝兆祥，侯乐峰 . 我国软籽石榴研究现状、存在问题及建议[J]. 中国果树，2017(1)：96-100.
LUO Hua, LIU Na, HAO Zhaoxiang, HOU Lefeng. Research, problems and suggestions of soft-seed pomegranate in China[J]. China Fruits, 2017(1): 96-100.

[46] 代正福，钟国富 . 名特优稀水果会理青皮软籽石榴[J]. 耕作与栽培，1999(1)：3-5.
DAI Zhengfu, ZHONG Guofu. Famous, special and rare fruit-*Punica granatum* L. Huiliqingpiruanzi[J]. Tillage and Cultivation, 1999(1): 3-5.

[47] 张敬富，陈思宇，陈织艳 . 淮北软籽石榴新品种选育[J]. 山西果树，2003(5)：41-42.
ZHANG Jingfu, CHEN Siyu, CHEN Zhiyan. Breeding of new pomegranate varieties with soft seed in Huaibei[J]. Shanxi Fruits, 2003(5): 41-42.

[48] 李斌 . 淮北软籽石榴品种及繁育技术[J]. 安徽林业，2006(4)：37.
LI Bin. Variety and breeding technique of Huaibei soft seed Pomegranate[J]. Anhui Forestry, 2006(4): 37.

[49] 朱立武，张水明，巩雪梅，贾兵，李绍稳，李瑶 . 软籽石榴新品种'红玉石籽'[J]. 园艺学报，2005，32(5)：200.
ZHU Liwu, ZHANG Shuiming, GONG Xuemei, JIA Bing, LI Shaowen, LI Yao. A new soft-seeded pomegranate variety 'Hongyushizi' [J]. Acta Horticulturae Sinica, 2005,32(5): 200.

[50] 赵国荣，朱立武，张水明，贾兵，李绍稳 . 软籽石榴新品种'红玛瑙籽'[J]. 园艺学报，2007，34(1)：260.
ZHAO Guorong, ZHU Liwu, ZHANG Shuiming, JIA Bing, LI Shaowen. A new soft-seeded pomegranate variety Hongmanaozi [J]. Acta Horticulturae Sinica, 2007,34(1): 260.

[51] 渠广林，渠利娜 . 范村软籽石榴及其栽培技术[J]. 落叶果树，2007(4)：27-28.
QU Guanglin, QU Lina. Soft-seeded pomegranate variety Fancunruanzi and its cultivation techniques[J]. Deciduous Fruits, 2007(4): 27-28.

[52] 叶振风，朱立武，张水明，贾兵，衡伟 . 软籽石榴新品种白玉石籽的选育[J]. 中国果树，2009(5)：12-13.
YE Zhenfeng, ZHU Liwu, ZHANG Shuiming, JIA Bing, HENG Wei. Breeding a new soft-seeded pomegranate variety Hongmanaozi[J]. China Fruits, 2009(5): 12-13.

[53] 安广池，刘桂平，闫志佩，王亮 . 软籽石榴新品种选育初报[J]. 中国园艺文摘，2010，26(7)：1-8.
AN Guangchi, LIU Guiping, YAN Zhipei, WANG Liang. A preliminary report on variety breeding of soft-seeded pomegranate[J]. Chinese Horticulture Abstracts, 2010, 26(7): 1-8.

[54] 郭俊英，曹尚银，薛华柏，刘丽 . 软籽石榴新品系中农红软籽石榴选育初报 [C]//中国园艺学会石榴分会 . 中国石榴研究进展(一). 北京：中国农业出版社，2010: 97-101.
GUO Junying, CAO Shangyin, XUE Huabai, LIUN Li. Soft-seed pomegranate strain-perliminary study on chinese agricultural soft-seed pomegranate [C]// Pomegranate Division of Chinese Society for Horticulture Science. The research progress of pomegranate (I). Beijing: China Agricuture Press, 2010: 97-101.

[55] 高福玲，李好先，陈利娜，牛娟，薛辉，张杰，赵弟广，曹尚银 . 石榴新品种'中石榴 2 号'的选育[J]. 果树学报，2017，34(7)：921-924.
GAO Fuling, LI Haoxian, CHEN Lina, NIU Juan, XUE Hui, ZHANG Jie, ZHAO Diguang, CAO Shangyin. A new pomegranate cultivar 'Zhongshiliu 2' [J]. Journal of Fruit Science, 2017, 34(7): 921-924.

[56] 李好先，曹尚银，姚方，骆翔，马贯羊，袁新征，夏小丛，赵弟广，常见，陈利娜 . 超软籽石榴新品种'慕乐'的培育[C]//曹尚银，李好先 . 中国石榴研究进展(三). 北京：中国林业出版社，2018: 147-151.
LI Haoxian, CAO Shangyin, YAO Fang, LUO Xiang, MA Guanyang, YUAN Xinzheng, XIA Xiaocong, ZHAO Diguang, CHANG Jian, CHEN Lina. A new super soft-seed pomegranate cultivar 'Mule' [C]//CAO Shangyin, LI Haoxian. The research progress of pomegranate (III). Beijing: China Forestry Publishing House, 2018: 147-151.

[57] 姚方 . 优良软籽石榴新品种玛丽斯[J]. 中国果业信息，2019，36(9)：55-56.
YAO Fang. A new soft-seeded pomegranate variety Malisi[J]. China Fruit News, 2019, 36(9): 55-56.

[58] 李好先，曹尚银，姚方，骆翔，辛长永，赵弟广，张淑英，夏小丛，刘军，陈利娜，姚海雷，敬丹 . 超软籽石榴新品种中石榴 8 号[J]. 园艺学报，2019，46(S2)：2772-2773.
LI Haoxian, CAO Shangyin, YAO Fang, LUO Xiang, XIN Changyong, ZHAO Diguang, ZHANG Shuying, XIA Xiaocong, LIU Jun, CHEN Lina, YAO Hailei, JING Dan. A new soft-seed pomegranate cultivar Zhongshiliu 8[J]. Acta Horticulturae Sinica, 2019, 46(S2): 2772-2773.