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枣和酸枣果实韧皮部糖分卸载途径及其积累研究

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摘 要:【目的】探索不同品种枣和酸枣果实同化物卸载途径差异及其糖分积累特点。【方法】以陕北制干枣品种'木枣'、兼用品种'狗头枣''邢台酸枣'和'清涧酸枣'为试验材料,利用透射电镜观察果实韧皮部卸载区细胞超微结构,用高效液相色谱(HPLC)测定不同发育时期枣果实果糖、葡萄糖和蔗糖含量。【结果】'木枣''狗头枣''邢台酸枣'和'清涧酸枣'果实鲜质量生长为双"S"型曲线,果实生长分为前期、中期和后期3个阶段;'木枣''狗头枣''邢台酸枣'和'清涧酸枣'果实不同发育阶段韧皮部筛管伴胞复合体与周围薄壁细胞间胞间连丝密度差异显著,'木枣''狗头枣'中期胞间连丝丰富,胞间连丝密度明显高于前期和后期;'邢台酸枣'前、中期有少量的胞间连丝,后期几乎观察不到胞间连丝;'清涧酸枣'后期仅有少量胞间连丝,前、中期几乎观察不到胞间连丝。'木枣''狗头枣'果实发育前期和中期主要积累果糖和葡萄糖,后期主要积累蔗糖。这与酸枣成熟期可溶性糖含量较低,糖积累不明显形成鲜明对比。【结论】不同品种枣果实同化物卸载途径一致,均经历前期质外体途径,中期以共质体途径为主,后期又转换为质外体卸载途径;而酸枣果实虽然存在少量胞间连丝,但总体以质外体运输途径为主。栽培枣的糖含量显著高于酸枣,共质体的运输方式对糖积累具有重要作用。

关键词:枣;酸枣;果实;同化物卸载;糖

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Phloem unloading and sugar accumulation in jujube fruits

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Abstract:【Objective】The transportation and distribution of photoassimilates is an important factor determining fruit yield and quality. Recently, the physiological mechanisms of photoassimilate unloading from sieve elements and phloem after transportation have been a heat subject in plant physiology and cell biology. Phloem unloading pathways, symplastic or apoplastic, may differ among plant species. Phloem unloading pathway in different organs of the same plant and in the same organ at different developmental stages, can be apoplasmic or symplasmic pathways or conversable between the two. Jujube is a very important economic horticultural crop, and its yield and quality is determined by sugar accumulation and composition in fruit. Phloem unloading is one of the key steps in accumulation of sugars in fruit. The phloem unloading pathways and their relationship to sugar accumulation remain unclear in Chinese jujube cultivars for various uses (dry processing, fresh consumption or multiple uses). In this study, we analyzed the unloading pathways in relation to sugar accumulation in fruits of different types of Chinese jujube.【Methods】Phloem unloading pathways and sugar contents were studied in cultivar 'Muzao' for drying processing, 'Goutouzao' for multiple uses, and two wild sour jujubes from Qingjian and Xingtai with electron microscopy and high performance liquid chromatography (HPLC). The fresh jujube fruits were cut into small

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pieces of 5 mm × 5 mm × 2 mm (length × width × height), quickly put into a serum bottle containing 2% glutaraldehyde, vacuum pumped for 40 min, fixed at 4 ℃ for 24 h, rinsed with 0.2 mol of phosphate buffer, and post-fixed with 1% osmium acid for 2 h at 4 ℃. After ethanol dehydration processes and propylene oxide transition, the samples were embedded with Spurr resin, polymerized at 68 ℃ for 8 h. The embedded blocks were shaped and sliced with an ultra-thin microtome into sections with thickness of 0.5 nm, which were stained with uranium acetate and lead citrate before observed under an HT7700 transmission electron microscopy. 1.0 g jujube powder was put into a 50 mL Conical flask with stopper, to which 40 mL 80% ethanol solution was added. After ultrasonic extraction for 20 min at 45 ℃, the suspension was centrifuged at freezing temperature and 3 500 g for 10 min. The supernatant was transferred to a 150 mL beaker, while the residue was re-suspended with 30 mL 80% ethanol solution. The extraction procedures were repeated twice, and the supernatants were pooled and rotary evaporated to dryness at 45 ℃, and then re-dissolved with 100 mL pure water. After filtration through a 0.22 μm filter membrane, analysis of sugar content was conducted using a HITACHI-2000 High Performance Liquid Chromatography. 【Results】The results showed that fresh weights of jujube and wild jujube fruits followed a "double S" curve, which could be divided into three stages: the early, the middle and the late stages. In the early stage, the relative growth rate of fruit was 3.50, which was highest in the whole growth period, with the fastest increase in fruit fresh weight and rapid cell division. In the middle stage, the fresh weight growth rate was 0.44. In the late stage, the fruit fresh weight growth rate was only 0.18. Structural investigations showed that plasmodesmata density in SE/CC complex and the surrounding parenchyma cells changed obviously. Plasmodesmata in 'Muzao' were observed between SE/CC complex and its surrounding phloem parenchyma cells in different developmental stages. In the middle stage, plasmodesmata density (2.48 μm^{-1}) was clearly higher than in the early and the late stages. There was no significant difference in plasmodesmata density between the early and the late stages. A large number of plasmodesmata in 'Muzao' were found in the early stage, its density was higher (1.07 μm^{-1}). With the fruit development, the plasmodesmata number gradually reduced to 0.71 μm^{-1} in the middle stage, and plasmodesmata were hardly observable in SE/CC complex in the late stage. There was no difference in plasmodesmata in phloem parenchyma cells in different developmental stage, and this pattern was also found in 'Goutouzao'. Some plasmodesmata were observed in the three developmental stages 'Goutouzao' in three tissues. Density of plasmodesmata between SE/CC complex and its surrounding parenchyma cells in different developmental stages was slightly lower than that in 'Muzao'. Plasmodesmata density was higher (0.68 μm^{-1}) in the late stage, but plasmodesmata between SE/CC complex and its surrounding phloem parenchyma cells were hardly observed in the early and the middle stages in sour jujube from Qingjian. Within SE/CC complex plasmodesmata were hardly observed in early stage, and their density was 0.78 μm^{-1} in middle stage but decreased gradually later. This trend was also observed in the sour jujube from Xingtai, where plasmodesmata density in the SE/CC complex reduced. There were more plasmodesmata between SE/CC complex and its surrounding phloem parenchyma cells the sour jujube from Xingtai in the early and the middle stages, but they were hardly observable in the late stage. However, in SE/CC complex, plasmodesmata were hardly observable in the early stage, but their density in middle stage reached a maximum of 0.98 μm^{-1} and then gradually decreased to 0.56 μm^{-1} in the late stage. There were numerous plasmodesmata in phloem parenchyma cells in different developmental stages in two wild jujubes. Fruit mainly accumulated glucose and fructose in the early and middle stages while accumulated sucrose mainly at the late stage. Fructose content in 'Goutouzao' increased first and then decreased to a relatively low level. The change in fructose content in 'Muzao'

showed an increasing trend. Glucose content showed a slow increasing trend during the development of 'Goutouzao', while in 'Muzao', glucose content fluctuated. Changes in sucrose content during fruit development were in a similar pattern in 'Goutouzao' and 'Muzao'. Previous results indicated that the soluble sugar content was lower in the wild jujube fruits.【Conclusion】These results suggested that there is no difference in phloem unloading pathways between jujube cultivars for different uses, whose phloem unloading follows apoplasmic-symplasmic-apoplasmic pathways. Although a small number of plasmodesmata were observed in different use of wild jujube, apoplasmic pathway might be dominant. In the aspect of sugar accumulation, in the early stage sucrose is unloaded by the apoplasmic pathway and decomposed into fructose and glucose in the phloem parenchyma. Therefore, glucose and fructose are accumulated in the early stage. In the middle stage, the sucrose unloading follows a symplasmic pathway through plasmodesmata, and the content of sucrose slowly increase. In the late stage, sucrose accumulates rapidly with the decrease in fructose and glucose. The three sugars in the wild jujubes are low throughout fruit development. Thus, sugar contents in jujube cultivars are significantly higher than in wild jujubes. High sugar accumulation might be related to symplasmic phloem unloading.

Key words: Chinese jujube; Wild jujube; Fruit; Phloem unloading of photoassimilate; Sugar

同化物的运输和分配是决定作物产量和品质的重要因素，同化物的转运是个十分复杂的过程。果实同化物从叶片合成后，大部分以蔗糖的形式经源叶韧皮部装载、韧皮部长距离运输、果实库韧皮部筛分子卸载、筛分子后运输等步骤，完成从源运输到库组织。果实库韧皮部筛分子卸载及筛分子后运输途径是2个密切联系的过程，对库器官物质运输和分配起关键作用[1]，成为果实库物质运输研究的热点。

对大型果实库来说，同化物韧皮部卸载是决定果实转运的重要步骤[2]。卸载是同化物经过长距离运输后的第一个步骤，指同化物从韧皮部筛管伴胞复合体（sieve element-companion cell，SE/CC）到周围韧皮薄壁细胞（parenchyma cell，PP）的过程，胞间连丝是细胞间共质体运输的通道，其数量多少与卸载方式有关，通过电子显微镜观察韧皮部各细胞间的胞间连丝，可以为同化物卸载机制提供依据[3]。同化物可通过共质体（symplasmic pathway）、质外体（apoplasmic pathway）或2者交替等3类卸载方式卸载，由于共质体途径有较高的转运能力和低运行阻力[4]，因此被认为是优于质外体转运途径。共质体和质外体卸载途径并不相互排斥，有的果实中2种卸载途径同时存在[5]。上个世纪80年代有学者利用"空种皮杯法"（empty seed coat technique）研究了豆科种子同化物的卸载方式[6]，近些年果实卸载途径研究主要利用荧光标记技术、超微电镜结构、激光共聚扫描显微镜、酸性转化酶的定位等技术。目前，果实库如苹果、梨、葡萄、番茄、核桃等卸载途径已有报道。苹果[7]、梨[8]等果实韧皮部组织与周围薄壁组织存在共质体隔离，同化物的卸出以质外体卸出为主；葡萄[9]、番茄[5]的卸载途径在果实发育的不同时期有所差别，均存在2种途径的转换，由发育前期的共质体途径转换为后期的质外体途径；核桃肉质果皮同化物卸载为质外体途径，种皮同化物卸载为共质体途径[10]；Nie等[11]对鲜食枣品种'冬枣'果实韧皮部研究发现，'冬枣'存在前、后期的质外体卸载方式与中期的共质体卸载方式。说明果实同化物卸载途径主要因果实种类或发育时期不同而不同。

果实糖分是果实品质的重要体现，果实积累糖的种类、含量对果实风味、色泽、营养有重要影响，是决定果实品质和商品价值的主要因素[12]。枣果实可溶性糖主要为蔗糖、果糖和葡萄糖，其中蔗糖含量最高，属蔗糖积累型[13]。果实中糖积累受到卸载路径的影响，如在苹果果实研究中，同化物卸载主要是质外体途径。质外空间主要含果糖和葡萄糖，而山梨醇和蔗糖的浓度均较低，这为质外体为主的同化物卸载机制提供了佐证。

枣是原产我国的特有果树，由酸枣起源驯化而来，枣果实含糖量高，而酸枣果实糖含量较低[14]。枣和酸枣糖含量差异很大，且品质参差不齐，它们卸载途径是否存在差异并不清楚。笔者以枣品种'木枣''狗头枣'以及'邢台酸枣'和'清涧酸枣'为材料，利用透射电镜和高效液相色谱法，通过超微结构观察

各发育阶段枣果实韧皮部及其周围薄壁细胞超微结构特征，研究枣果实发育不同时期的糖分积累特点，阐明枣果实发育结构与果实糖分积累和运输的关系提供细胞学依据，为枣品质形成机制和生产上枣品质提升提供理论依据。

1　材料和方法

1.1　材料

试验材料采自西北农林科技大学清涧红枣试验站。选择5 a生健康‘木枣’‘狗头枣’‘邢台酸枣’和‘清涧酸枣’树各6株，生长情况基本一致，管理措施相同。在盛花期（6月10日）挂牌标记同一发育时期的花，对应这些花的果实为试验用果。采样从2013年6月28日开始（盛花期后20 d）到2013年9月25日（盛花期后110 d）结束，分别选幼果期（盛花期后25~30 d）、膨大期（盛花期后55~60 d）、白熟期（盛花期后70~80 d）、半红期（盛花期后90~100 d）、全红期（盛花期后100~110 d）的果实进行采摘。

每株树冠东南西北4个方位随机采集各发育阶段的健康果实5~10个，用刀片将新鲜枣果果皮削去，将靠近果皮的果肉组织切成5 mm×5 mm×2 mm小块（5块），迅速放入装有2%戊二醛血清瓶中，待用。

1.2　方法

1.2.1　*果实鲜质量测定*　每次采样在每株树冠东南西北4个方位采集挂牌果，每个品种各采集20个果，采后迅速拿回实验室用电子天平称量并记录（单位g）。

1.2.2　*电镜超微制片*　组织包埋块制备参考吕英民等[15]的方法，稍有改动。每株树冠东南西北4个方位随机采集各个发育阶段的健康果实5~10粒，用手术刀片将新鲜枣果果皮削去，将靠近果皮的果肉组织切成5 mm×5 mm×2 mm（长×宽×高）小块（5块），迅速投入装有2%戊二醛血清瓶中，真空抽气40 min，在4 ℃固定24 h。然后用0.2 mol·L^{-1}磷酸缓冲液冲洗，1%锇酸固定2 h，4 ℃系列乙醇脱水、环氧丙烷过渡、Spurr树脂包埋，在68 ℃下聚合8 h。修块后用ULTRACUT型超薄切片机切片，厚度50 nm，醋酸双氧铀和柠檬酸铅染色，日立HT7700型透射电镜观察拍照。6月28日采样1次；8月10日开始每隔10 d采样1次。

胞间连丝数量测定参考吕英民等[16]的方法，有所改动。对每一时期维管束包埋材料做5次连续切片，每一次连续系列切片和上次系列切片之间大约相距40 μm，随机捞取5条样品带，染色后在电镜下拍照观察。统计维管束横切面上SE/CC，SE/PP和CC/PP，PP/PP细胞界面上的胞间连丝数量，并计算细胞界面平均长度，以胞间连丝数量与细胞界面平均长度比值作为胞间连丝密度（每μm个数）测定值。根据果实鲜质量生长曲线，将果实分为3个时期，将每时期内所有胞间连丝密度求平均值作为时期的胞间连丝平均值。

1.2.3　*糖提取和含量测定*　提取参照Gao等[17]的方法，稍有改动。准确称取1.0 g枣粉于50 mL具塞三角锥形瓶中，加入40 mL 80%乙醇溶液，45 ℃超声提取20 min，使可溶性糖充分浸出，冷却后3 500 g冷冻离心10 min，将上清液转入150 mL烧杯中；再向残渣中加入30 mL 80%乙醇溶液，重复浸提取2次，合并上清液，45 ℃旋转蒸干，超纯水定容至10 mL。用0.22 μm滤膜过滤后待测。生物学重复为3组。

采用日立-2000高效液相色谱仪进行糖含量分析。糖测定色谱条件：氨基柱，柱温35℃，流动相$V_{乙腈}:V_{水}$=80∶20，流速1 mL·min^{-1}，检测器为示差折光检测器，检测池温度35 ℃，进样体积10 μL，外标法定量。标样为Sigma公司产品，其他试剂均为色谱纯级。

1.3　数据分析

利用SPSS和Excel进行数据分析和作图。

2　结果与分析

2.1　果实生长曲线

木枣果实鲜质量生长曲线呈双S型，如图1所示。前期（花后0~30 d），果实相对生长速率值为3.50，为整个生长期最大，果实鲜质量变化最快，果实细胞快速分裂；中期（花后31~80 d），鲜质量生长速率均为0.44，相对于前期鲜质量变化缓慢；III期，即后期（花后81~110 d），鲜质量平均生长速率为0.18，鲜质量增长很缓慢，果实主要进行营养物质的积累和转化。果实生长阶段的划分便于卸载途径的研究，‘狗头枣’与‘木枣’‘邢台酸枣’‘清涧酸枣’鲜质量变化趋势基本一致。

2.2　果实韧皮部超微结构观察

枣和酸枣果实不同发育时期韧皮部各细胞超微结构如图2~图4所示。可以看出，韧皮部主要有筛

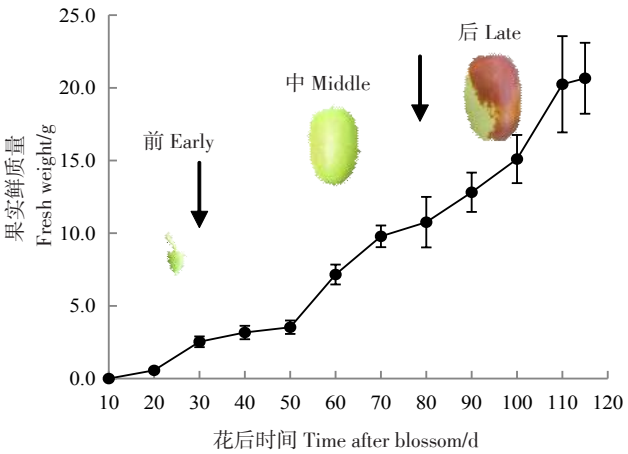
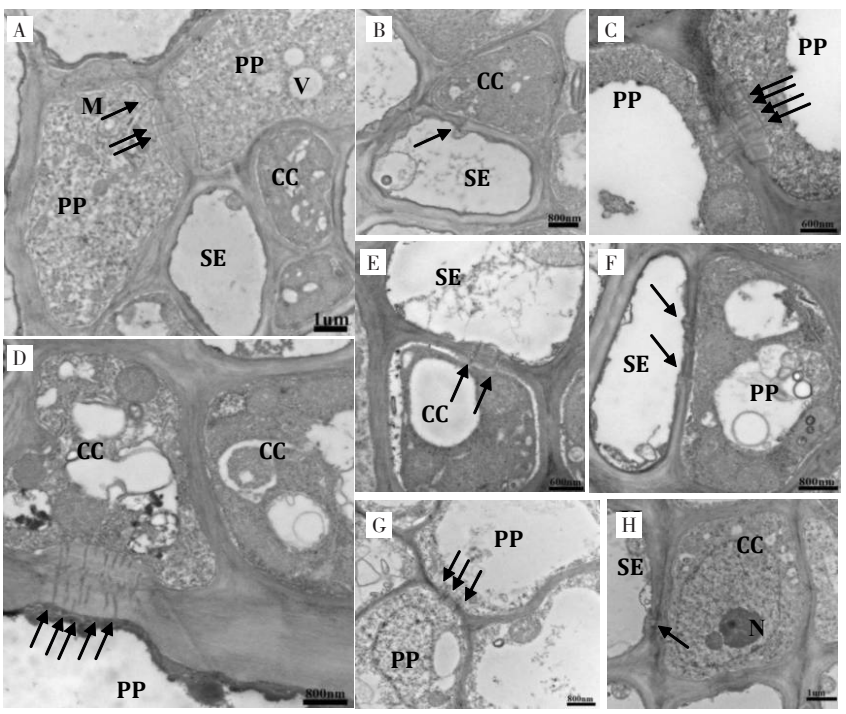


图 1 ‘木枣’果实鲜质量的变化

Fig. 1 Changes of fresh weight of ‘Muzao’

分子、伴胞和韧皮薄壁细胞。筛分子常呈梯形，内部被一中央大液泡占据，少量原生质紧贴在细胞壁内侧（图2-B），不同发育时期筛分子腔内存在不同形态的丝状不定形物质（图2-B、E），筛分子腔内可见黑色颗粒状黏液体（图4-C、G），筛分子之间存在筛板（图3-E）。伴胞和筛分子大小相似，多呈三角形，胞质稠密，染色深，细胞核明显（图2-B、H），含有线粒体等多种细胞器，在发育中期可见清晰的线粒体内部结构（图2-D~E；图3-C）。常常一个或多个邻接在筛分子旁形成筛管伴胞复合体，筛分子与伴胞间有胞间连丝（图2-B、E；图3-F；图4-C）。薄壁细胞其面积一般比筛管和伴胞大，呈不规则椭圆形（图4-A），染色较浅，含有多种细胞器如线粒体、内质网、高尔基体等在整个果实发育期间薄壁细胞间均有丰富的胞间连丝（图2-A、C、G；图3-C；图4-A、F）。

韧皮部不同细胞间胞间连丝密度如表1、2所

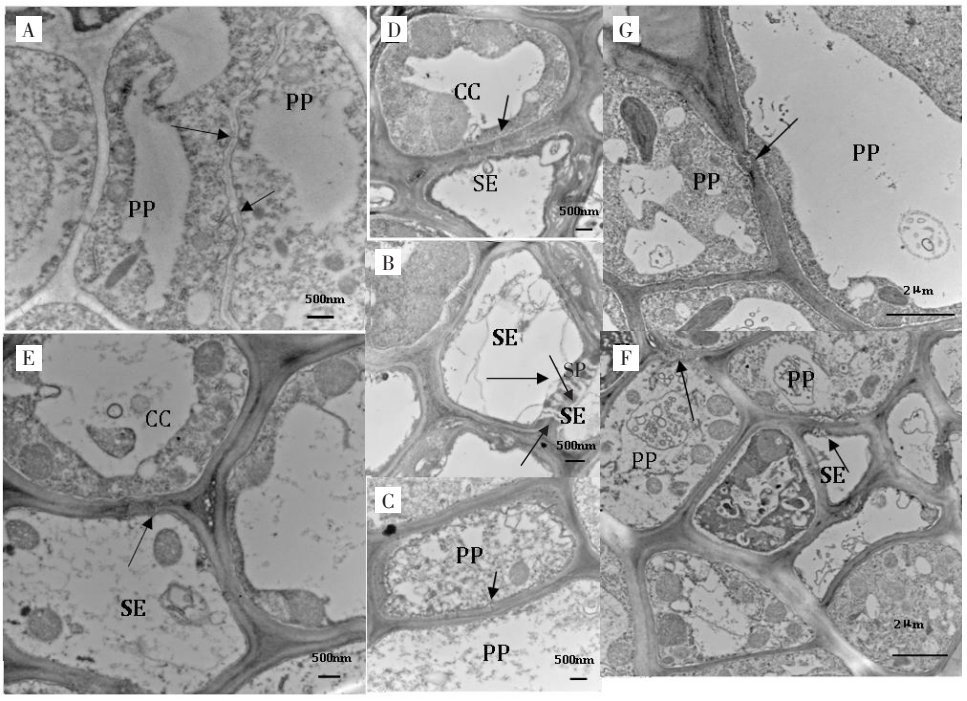


A~B. 花后 30 d 果实韧皮部超微结构。A. PP 间胞间连丝和 PP 内线粒体和液泡；B. 筛管伴胞复合体，箭头示 SE 与 CC 间胞间连丝；C~F. 花后 60 d 果实韧皮部超微结构，见各细胞间胞间连丝，C 箭头示 PP 间胞间连丝，D 箭头示 CC 与 PP 间丰富的胞间连丝，E 示 SE 和 CC 胞间连丝，F 示 SE 和 PP 间胞间连丝。G~H. 花后 100 d 果实韧皮部超微结构。G 箭头示 PP 间胞间连丝，H 箭头示 SE 与 CC 间胞间连丝，CC 内明显细胞核。SE. 筛分子；CC. 伴胞；PP. 韧皮部薄壁细胞；M. 线粒体；V. 液泡；N. 细胞核。

A-B. The ultrastructure of fruit phloem 30 d after full bloom; A . Plamodesmata between PPs and mitochondria and vacuole; B. A SE-CC complex and plamodesma between them. C-F. The ultrastructure of fruit phloem 60 d after full bloom; C . Plamodesmata between PPs; D. A number of plamodesmata between CC and PP; E. Plamodesmata between SE and CC; F. Plamodesmata between SE and PP. G-H. The ultrastructure of fruit phloem 110 d after full bloom; G. Plamodesmata between PPs; H. Plamodesma between SE and CC, a clear nucleus in CC. SE. Sieve element; CC. Companion cell; PP. Phloem parenchyma cell; M. Mitochondrion; V. Vacuole; N. Nucleus.

图 2 ‘木枣’果实韧皮部细胞超微结构

Fig. 2 The ultrastructure of phloem cells of ‘Muzao’ fleshy fruit



A. 花后 30 d 果实韧皮部超微结构，A 示 PP 间胞间连丝和 PP 内线粒体和液泡。B~D. 花后 60 d 果实韧皮部超微结构，示各细胞间胞间连丝，B. 箭头示 SE 与 CC 间胞间连丝；C. 箭头示 PP 间胞间连丝；D. 筛管伴胞复合体，箭头示 SE 与 CC 间胞间连丝。E~G. 花后 100 d 果实韧皮部超微结构，示各细胞间胞间连丝，E. 筛分子之间的细胞壁形成筛板，F 箭头示 SE 与 PP 间的胞间连丝及 PP 间的胞间连丝，G 箭头示 PP 间丰富的胞间连丝。SE. 筛分子；CC. 伴胞；PP. 韧皮部薄壁细胞；SP. 筛板。

A. The ultrastructure of fruit phloem 30 d after full bloom, A shows plamodesmata between PPs and mitochondria and vacuole. B-D. The ultrastructure of fruit phloem 60 d after full bloom; B Plamodesmata between SE and CC; C. Plamodesmata between PPs; D. A SE-CC complex and plamodesma between them. E-H. The ultrastructure of fruit phloem 100 d after full bloom; E. The sieve plate in the SE cell wall; F. Plamodesmata between SE and PP and plamodesmata between PPs; G. A number of plamodesmata between PPs. SE. Sieve element; CC. Companion cell; PP. Phloem parenchyma cell; SP. Sieve plate.

图 3　‘清涧酸枣’果实韧皮部细胞超微结构

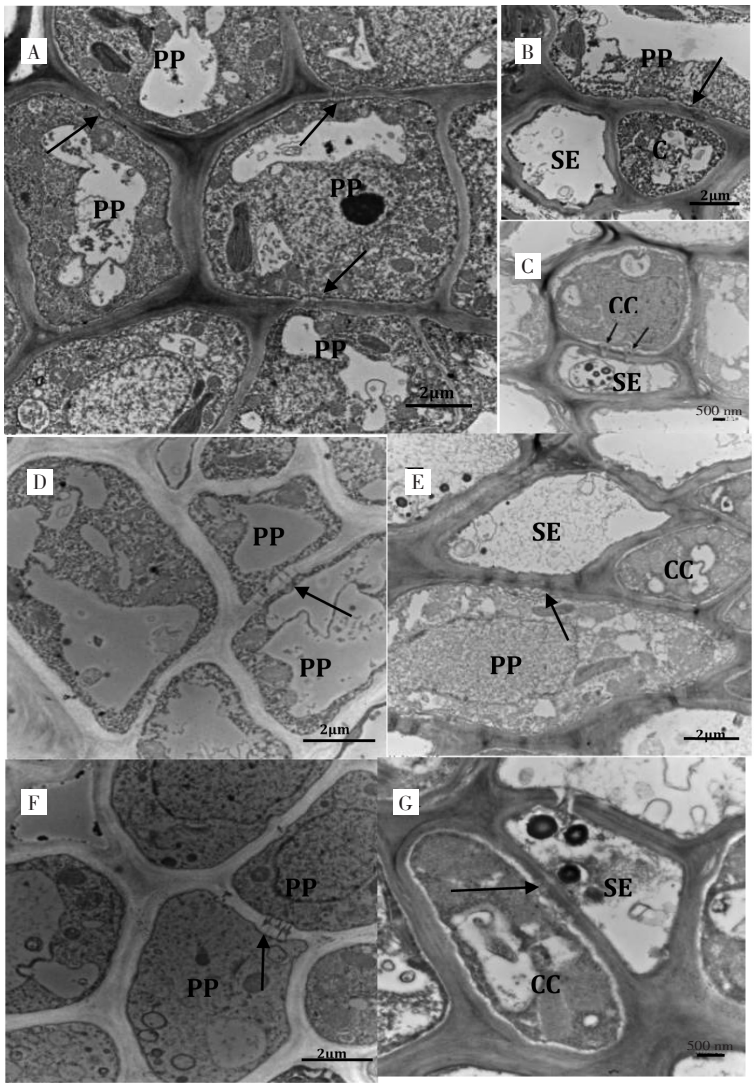
Fig. 3 The ultrastructure of phloem cells of ‘Qingjian Suanzao’ fleshy fruit

示。‘木枣’和‘狗头枣’韧皮部薄壁细胞间存在丰富的胞间连丝，胞间连丝密度在整个发育时期都较高；筛管与伴胞间胞间连丝随果实发育逐渐减少；筛管伴胞复合体与薄壁细胞间胞间连丝在中期最大。

‘木枣’不同发育时期筛管与伴胞之间、筛管伴胞复合体与韧皮薄壁细胞之间胞间连丝密度差异显著，但韧皮薄壁细胞之间胞间连丝密度无差异。‘木枣’果实发育前期胞间连丝丰富，胞间连丝密度值较高（每 μm 1.07 个），随着果实发育胞间连丝数量逐渐减少，中期胞间连丝密度为每 μm 0.71 个，后期筛管伴胞间几乎观察不到胞间连丝。‘木枣’筛管伴胞复合体与薄壁细胞间在不同发育阶段均可观察到胞间连丝，但中期胞间连丝密度明显大于前期和后期，分别为前期和后期4倍和3倍，前期和后期密度无显著差异，约为每 μm 0.70 个。‘狗头枣’筛管与伴胞、筛管伴胞与薄壁细胞间胞间连丝在不同发育时期差异显著，薄壁细胞间胞间连丝在不同时期差异不显著，与‘木枣’一致；狗头枣果实前期、中期和后期筛管伴胞间均观察到了一定数量的胞间连丝，但胞间连丝数量随发育逐渐减少；不同时期‘狗头枣’筛管伴胞与薄壁细胞间胞间连丝数量均略低于‘木枣’。

‘清涧酸枣’筛管与伴胞胞间连丝密度在前、中、后3个发育时期有所差异。果实发育前期几乎观察不到胞间连丝，中期胞间连丝密度为每 μm 0.78 个，后期胞间连丝密度略微降低（每 μm 0.65 个）。筛管伴胞复合体与薄壁细胞间的胞间连丝密度差异显著，前中期几乎观察不到胞间连丝，后期胞间连丝密度升高为每 μm 0.68 个。不同发育时期薄壁细胞间均存在丰富的胞间连丝，以中后期最为明显，胞间连丝密度均在每 μm 1.00 个以上，但差异不显著。

‘邢台酸枣’筛管伴胞间不同时期胞间连丝差异显著，前期几乎观察不到胞间连丝，中期胞间连丝密



A~B. 花后 30 d 果实韧皮部超微结构，A 示 PP 间胞间连丝。B 箭头示 CC 与 PP 间的胞间连丝 . C~E. 花后 60 d 果实韧皮部超微结构，示各细胞间胞间连丝，C 筛管伴胞复合体，箭头示 SE 与 CC 间胞间连丝，D 箭头示 PP 间胞间连丝，E 箭头示 SE 与 PP 间的胞间连丝。F~G. 花后 100 d 果实韧皮部超微结构，示各细胞间胞间连丝，F 箭头示 PP 间胞间连丝，G 箭头示 SE 与 CC 间的胞间连丝。SE. 筛分子；CC. 伴胞；PP. 皮部薄壁细胞。

A–B. The ultrastructure of fruit phloem 30 d after full bloom; A shows plamodesmata between PPs, B shows plamodesmata between CC and PP. C–E. The ultrastructure of fruit phloem 60 d after full bloom; C shows a SE–CC complex and plamodesma between them, D shows plamodesmata between PPs, E shows plamodesmata between SE and PP. F–G. The ultrastructure of fruit phloem 100 d after full bloom; F shows plamodesmata between PPs, G shows a SE–CC complex and plamodesma between them. SE. Sieve element; CC. Companion cell; PP. Phloem parenchyma cell.

图 4 ‘邢台酸枣’果实韧皮部细胞超微结构

Fig. 4 The ultrastructure of phloem cells of ‘Xingtai Suanzao’ fleshy fruit

度达到最大（每 μm 0.98 个），后期又逐渐减少至每 μm 0.56 个。筛管伴胞复合体与薄壁细胞间的胞间连丝密度差异显著，前中期有较丰富的胞间连丝，密度最大（每 μm 1.89 个），而后期几乎观察不到胞间连丝。不同发育时期薄壁细胞间均有丰富的胞间连丝，以前、后期最为明显，胞间连丝密度约为每 μm 1.00 个，但差异不显著。

‘清涧酸枣’筛管和伴胞间的胞间连丝密度的变化趋势与‘邢台酸枣’类似，均是前期几乎观察不到胞间连丝，而中期胞间连丝都达到最大值，后期均有不同程度的减少。筛管伴胞复合体与薄壁细胞间胞间连丝密度的变化，在‘清涧酸枣’和‘邢台酸枣’中差异较大，‘清涧酸枣’的前、中期几乎观察不到胞间连丝，后期出现胞间连丝，而在‘邢台酸枣’中胞间连丝密度在前、中期就达到最大值，后期消失，几乎观察不到。‘清涧酸枣’和‘邢台酸枣’薄壁细胞间胞间

表1　‘木枣’和‘狗头枣’果实韧皮部细胞间胞间连丝密度

Table 1 Plasmodesmal densities between different cells in the developing *Z. jujuba* Mill. ‘Muzao’ and ‘Goutouzao’

（No·μm^{-1}）

发育时期 Stage	SE/CC		SE(CC) / PP		PP/PP	
	木枣 Muzao	狗头枣 Goutouzao	木枣 Muzao	狗头枣 Goutouzao	木枣 Muzao	狗头枣 Goutouzao
前期 Early	1.07	1.04	0.65	0.56	1.04	1.38
中期 Middle	0.71	0.62	2.48	2.08	1.53	1.48
后期 Late	0.00	0.43	0.74	0.65	1.98	0.85

注：SE. 筛分子；CC. 伴胞；PP. 皮部薄壁细胞。下同。

Note: SE. Sieve element; CC. Companion cell; PP. Phloem parenchyma cell. The same below.

表2　‘邢台酸枣’和‘清涧酸枣’果实韧皮部细胞间胞间连丝密度

Table 2 Plasmodesmal densities between different cells in the developing wild jujube（‘Xingtai Suanzao’ and ‘Qingjian Suanzao’）

（No·μm^{-1}）

发育时期 Stage	SE/CC		SE(CC) / PP		PP/PP	
	邢台酸枣 Xingtai Suanzao	清涧酸枣 Qingjian Suanzao	邢台酸枣 Xingtai Suanzao	清涧酸枣 Qingjian Suanzao	邢台酸枣 Xingtai Suanzao	清涧酸枣 Qingjian Suanzao
前期 Early	0.00	0.00	1.03	0.00	1.07	0.63
中期 Middle	0.98	0.78	1.89	0.00	0.88	1.00
后期 Late	0.56	0.65	0.00	0.68	1.18	1.35

连丝密度差异不显著，各个时期均有一定量的胞间连丝。

2.3　不同时期果实糖积累

笔者测定了‘木枣’‘狗头枣’不同发育成熟阶段果实糖含量。酸枣总体含糖量较低，此处引用张春梅[18]所测‘清涧酸枣’果实糖含量数据作为比较（图5）。‘木枣’和‘狗头枣’果实总糖均呈增加趋势；‘清涧酸枣’总糖含量较低，在发育过程中基本保持稳定。

‘狗头枣’果糖含量变化呈现先升高、后降低，进而趋于稳定的态势，在幼果期果实果糖质量分数较低（17.50 g·kg^{-1}），随后果糖质量分数增加，在白熟期达到最大值（64.50 g·kg^{-1}），随后果糖质量分数下降至42.28 g·kg^{-1}，并保持相对稳定；‘木枣’果糖质量分数变化整体呈增加趋势，幼果期糖质量分数较‘狗头枣’高（36.19 g·kg^{-1}），随后略微下降（25.78 g·kg^{-1}），后升高，到果实成熟时期（半红期）达最大（57.95 g·kg^{-1}），之后基本保持稳定；‘清涧酸枣’的果糖质量分数未出现较大范围的变化，生长发育阶段基本稳定在20 g·kg^{-1}以内。

‘狗头枣’葡萄糖含量在不同发育阶段总体呈现出缓慢增长的趋势，幼果期质量分数为41.55 g·kg^{-1}，膨大期略微下降至34.69 g·kg^{-1}，随后升高，到全红期

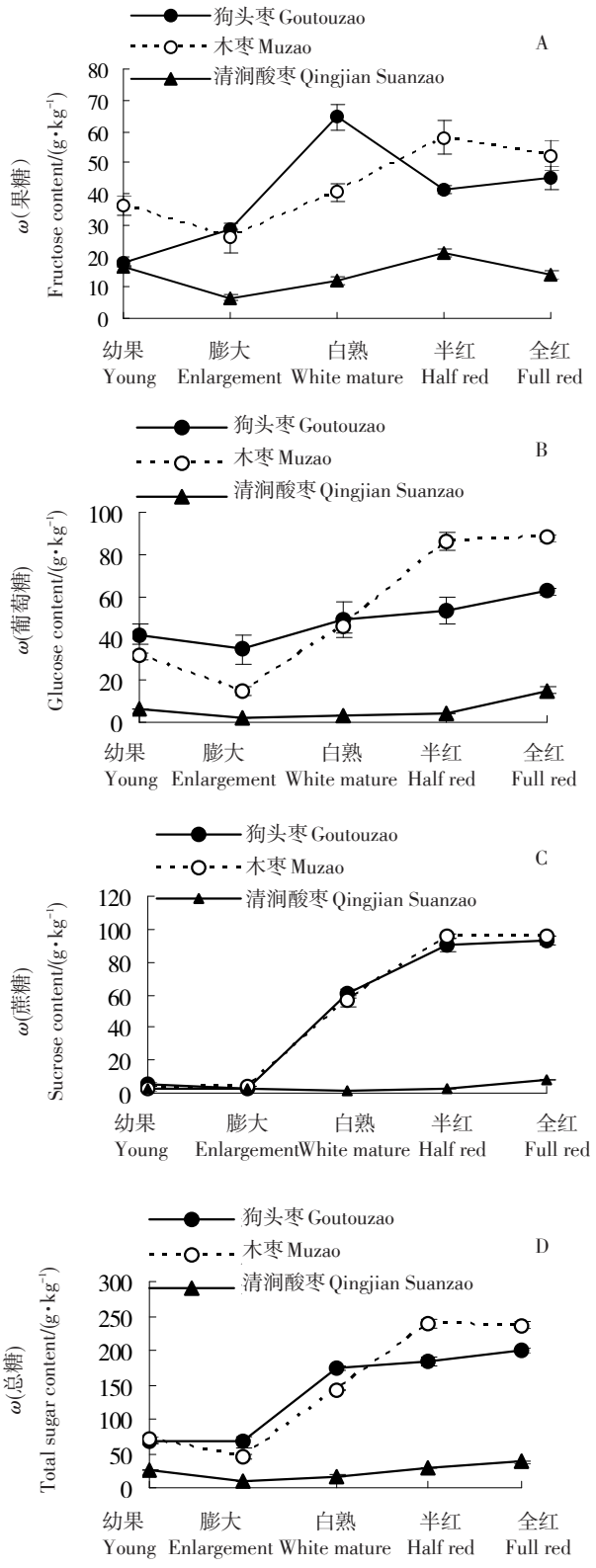


图5　果实发育过程中蔗糖、果糖、葡萄糖和总糖含量变化

Fig. 5 Changes in sugar contents in jujube fruit during development

达到最大(62.41 g·kg^{-1});'木枣'葡萄糖质量分数变化曲折,幼果期至膨大期质量分数由31.414 g·kg^{-1}下降至14.66 g·kg^{-1},随后呈现出迅速升高的状态,到半红期达到最大值(86.10 g·kg^{-1})后保持稳定;'清涧酸枣'的葡萄糖质量分数在发育阶段未出现较为明显的波动,基本保持在1~5 g·kg^{-1}。

'狗头枣'和'木枣'蔗糖质量分数在不同发育阶段变化趋势基本一致,幼果期至膨大期质量分数基本保持稳定,随后质量分数迅速升高,在全红期达到最大值(分别为92.51和95.74 g·kg^{-1})。'清涧酸枣'蔗糖质量分数在整个发育阶段均处于很低水平。

3 讨 论

枣果实生长发育呈双S型曲线,果实生长期分为前期、中期和后期3个阶段。前期果实细胞迅速分裂,果实鲜质量增加快;中期果实鲜质量开始增加、增速较快,果实细胞体积不断膨大,果实形状形成后营养物质开始积累,鲜质量变化速率低;后期果实鲜质量基本保持稳定,果实内糖分迅速积累,与其他有机物相互转化形成果实风味。

果实库卸载通过共质体、质外体或2者交替方式进行。共质体卸载方式主要通过胞间连丝进行[19],筛管伴胞复合体与周围韧皮薄壁细胞间有大量胞间连丝,卸载一般以共质体方式为主,并在马铃薯块茎[20]、番茄[5]等果实上得到证实。胞间连丝是细胞间物质运输与信息传递的重要通道,同时对运输有精确的调控[21],一般情况下,可根据韧皮部筛管-伴胞复合体与周围薄壁细胞界面上胞间连丝的分布以及频率,确定同化物卸载途径类型。利用羧基荧光素示踪技术、C-糖放射自显影技术以及酸性转化酶等技术在苹果、蓝莓[22]、胡桃、葡萄等果实发育阶段已经对此进行了验证。'木枣'和'狗头枣'果实发育中期筛管伴胞复合体与周围薄壁细胞间胞间连丝丰富,胞间连丝密度高。因此,确认'木枣'和'狗头枣'果实在中期以共质体卸载为主;而前期和后期筛管伴胞复合体与周围薄壁细胞间胞间连丝数量少,胞间连丝密度很低,说明枣果实发育前期和后期筛管伴胞复合体与周围的薄壁细胞不存在组织学上的联系,所以枣果实前期和后期以质外体卸载为主,与'冬枣'的卸载途径一致[3]。因此。说明枣果实同化物卸载都经历前期质外体-中期以共质体为主-后期质外体的转换卸载途径。

酸枣果实发育的各个时期,SE/CC复合体与薄壁细胞之间胞间连丝密度明显不同,'清涧酸枣'筛管伴胞复合体与薄壁细胞间的胞间连丝在前中期几乎观察不到,后期只有少量胞间连丝,其密度和枣相近。所以,说明'清涧酸枣'前、中期筛管伴胞复合体与薄壁细胞间不存在组织学上的联系,以质外体卸载途径为主。'邢台酸枣'筛管伴胞复合体与薄壁细胞间在前、中期有较丰富的胞间连丝,但多数堵塞;而后期几乎观察不到胞间连丝。胞间连丝作为共质体运输的标志,其通透性大小可以反映共质体的运输能力,对共质体运输有调节作用[23]。这说明'邢台酸枣'中期共质体运输能力较弱。

库卸载机制与库的发育和功能紧密相连,是一个动态过程。枣果实糖卸载的调控机制可以更进一步理解枣果实糖韧皮部卸载这一现象,枣果实糖卸载有2种途径:共质途径和质外体途径。因此,调控果实糖卸载就体现在这2种途径上。

枣果实发育前期糖以质外体途径卸载,蔗糖跨膜运输从筛管卸载到质外体空间,这是需要能量的主动运输。在质外体卸载过程中,蔗糖被细胞壁酸性转化酶分解为果糖和葡萄糖经质外体空间进入韧皮薄壁细胞[11],薄壁细胞内液泡酸性转化酶活性低,蔗糖合成量少,主要以果糖和葡萄糖形式存在。因此,在发育前期检测到较高浓度的果糖和葡萄糖;果实发育中期蔗糖进行共质体途径卸载,实质就是同化物通过胞间连丝卸载[24],导致蔗糖含量缓慢增加。后期枣果实内蔗糖迅速积累,果糖和葡萄糖含量上升,可溶性糖含量高,果实细胞渗透压大于运输组织渗透压,如果利用渗透压推动的共质体卸载方式会使营养物质从库细胞反流到运输细胞[1-2],而质外体途径主要依靠细胞膜载体和能量,各部分相互隔离从而阻碍运输物质反流,有效提高了成熟期营养物质进入库细胞。与枣相比,酸枣果实含糖量较低[18],其胞间连丝密度尤其在SE/PP之间明显低于枣。说明酸枣的低含糖量与其弱的糖卸载能力相关。

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