无花果果实韧皮部卸载路径由共质体向质外体途径转变

李春丽^①, 侯柄竹^②, 张晓燕^③, 李疆^①, 沈元月^{②*}

① 新疆农业大学林学与园艺学院, 乌鲁木齐 830052;

② 农业应用新技术北京市重点实验室, 北京农学院植物科学技术学院, 北京 102206;

③ 中国农业大学生物学院, 北京 100094

* 联系人, E-mail: sfmn@tom.com

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摘要 为了揭示无花果果实同化物韧皮部卸载机制, 以玛斯义陶芬无花果5个时期果实为试材利用透射电子显微镜结合荧光染料示踪技术研究同化物韧皮部卸载路径的变化. 无花果果实发育分3个时期, 第1个快速生长期、缓慢生长期、第2个快速生长期, 第2个生长期和第3个生长期之间为果实发育的转折期. 超微结构观察表明, 无花果始熟期前筛分子伴胞复合体和周围薄壁细胞存在共质体联系, 但始熟期后变为共质体隔离, 同时在果实整个发育过程中薄壁细胞间始终存在大量胞间连丝. 羧基荧光素(CF)引入表明, 始熟期前荧光染料从韧皮部卸出到周围薄壁细胞中, 在始熟期后CF被严格限制在韧皮部没有卸出到周围薄壁细胞. 结果表明, 无花果果实始熟期是同化物韧皮部卸载由共质体向质外体转变的转折期, 但同时韧皮部后运输始终为共质体途径.

关键词 无花果, 韧皮部卸载, 超微结构, 荧光示踪, 共质体, 质外体

无花果(*Ficus carica* L., Moraceae)属于桑科、亚热带落叶果树, 在我国新疆和山东沿海地区广泛栽培. 近年来, 研究者们把无花果引入北京并设施栽培取得成功. 无花果在设施栽培模式下抗病虫害、抗旱及果品高产优质, 具有重要经济价值和生态价值. 尽管无花果是人类驯化最早的经济作物^[1,2], 但无花果果实糖卸载的细胞学途径目前还不清楚.

韧皮部卸载是指同化物经韧皮部筛分子伴胞复合体卸出到邻近生长或储存库组织的过程, 对作物产量和品质起着决定性作用^[3-5]. 植物库器官韧皮部卸载途径主要通过共质体途径、质外体途径^[6-10]. 值得关注的是, 近几年来果实韧皮部的卸载引起了极大的关注并取得了显著的研究进展, 揭示了一批果

实糖卸载的途径及其卸载机制, 包括苹果(*Malus pumila*)^[11]、核桃(*Juglans regia*)^[12]、葡萄(*Vitis vinifera*)^[13]、枣(*Ziziphus jujuba*)^[14]、草莓(*Fragaria ananassa*)^[15]、番茄(*Lycopersicon esculentum*)^[16]、黄瓜(*Cucumis sativus*)^[17], 发现了苹果、黄瓜、草莓韧皮部卸载为质外体途径; 葡萄、番茄发育前期为共质体途径, 后期为质外体途径; 枣发育前期后期为共质体途径, 发育中期为质外体途径; 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. 以上研究结果揭示了光合同化物的卸载随着果实类型和果实发育时期而改变.

本研究采用透射电子显微镜超微结构观察结合荧光染料示踪技术首次对无花果果实从花芽分化结

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束后5个发育时期韧皮部同化物卸载方式变化过程进行了研究,揭示了无花果果实韧皮部卸载路径由共质体向质外体途径转变的细胞学途径,为无花果果实品质的改善奠定了研究基础.

1 材料与方法

(i) 材料. 供试材料来自于北京农学院试验田大棚内种植的玛斯义陶芬(Masui Dauphine)无花果果树,树龄3年,树体健壮. 实验于2014年6~8月无花果果期进行. 果实花芽分化结束后,即花托内出现小花开始^[18],直到果实成熟,每周取样两次,记录果实纵横径,绘制果实生长发育曲线. 根据其生长发育曲线,分别选取花芽分化结束后15(小绿果),30(大绿果),65(始红果),70(片红果),85 d(全红果)样品进行实验(图1).

(ii) 可溶性糖和淀粉含量测定. 可溶性糖含量参照李春丽等人^[19]的方法测定,淀粉含量用高氯酸水解蒽酮比色法测定.

(iii) 维管束分布. 参照Drazeta等人^[20]的方法测定. 1%的水溶性酸性品红溶液染料示踪法观察维管束的分布和运输,为了防止气泡栓塞木质部,用蒸馏水瓶浸没果实果柄基部,从果柄基部连接茎干处剪断,然后断口浸入染液,在室温下浸染2 h. 果实用刀片从赤道面切开,尽量减少组织损伤,使切面平滑.

(iv) 石蜡切片的制作. 随机采摘小绿果、大绿果、始红果、片红果、全红果5个时期果实,采后立即用锋利刀片分割,分别取0.5 cm×0.5 cm×1.5 cm果实(取样部位见图3(b)),立即投入FAA固定液(formalin-acetic acid-alcohol,福尔马林-醋酸-酒精固定液)中,室温固定24 h后漂洗、脱水、透明、浸蜡、包埋、修块,用轮转式切片机(YD-2508A,中国,上海珂淮仪器有限公司)切片,切片厚度为10 μm;番红固绿对染,中性树脂封片,数码显微镜(SZ2-ILST, Olympus, 日本)观察拍摄记录样品.

(v) 样品超微结构透射电子显微镜观察制片. 参照张凌云等人^[11]的方法,随机采摘小绿果、大绿果、始红果、片红果、全红果5个时期果实,采后立即用锋利刀片分割,分别取2 mm×2 mm×5 mm主要维管束部位(采样部位见图3(b)),3%戊二醛(0.2 mol L⁻¹磷酸缓冲液50 mL,25%戊二醛12 mL,加水定容至100 mL)固定,真空脱气,固定24 h后用0.1 mol L⁻¹的磷酸盐缓冲液(pH 7.2)洗涤样品10次,每次5 min;倒净清洗液后在通风橱中迅速加入1%四氧化钨固定

液没过样品,并立刻盖好盖,室温固定3 h,期间轻轻晃动样品一次;用0.1 mol L⁻¹的磷酸盐缓冲液(pH 7.2)洗涤样品10次,每次5 min;然后乙醇(30%, 50%, 70%, 80%, 90%, 95%, 100%, 100%)逐级梯度脱水,每级脱水15 min;之后用100%丙酮室温置换3次,每次15 min;分别用Super包埋剂:丙酮(1:3, 1:2, 1:1),100%Super包埋剂35℃各浸透8 h;将样品小心转移入包埋板孔中,调整位置后60℃烤箱烤15 h,取出样品块后修块,在超薄切片机(EM UC6, LEICA, 日本)上切片,切片厚度60 nm,用200/目铜网捞片,醋酸铀-柠檬酸铅双重染色,用透射电子显微镜(H-7650, HITACHI, 日本)观察拍照.

(vi) 胞间连丝密度测定. 参照Zhang等人^[11]和Kempers等人^[21]的方法测定. 用超薄切片机将各个时期样品包埋块做5次连续切片,每次连续切片和上次连续切片间大约相距20 μm,每次切片随机捞取6条样品带于2个0.25% Formvar膜覆盖的200目铜网上. 透射电子显微镜观察,分别选取4个筛分子/伴胞(sieve element/companion cell, SE/CC)复合体为中心的视野,统计SE/CC,筛分子/薄壁细胞(sieve element/parenchyma cell, SE/PC),伴胞/薄壁细胞(companion cell/parenchyma cell, CC/PC),薄壁细胞之间(PC/PC)的细胞界面的胞间连丝密度,胞间连丝密度是韧皮部横切面上细胞界面平均细胞壁长度上的胞间连丝个数. 所有的分支胞间连丝按照1个统计.

(vii) CFDA和Texas red标记. 参照Zhang等人^[11]的方法测定,选择健康向阳的果实,将果柄冲洗干净后,将棉线穿过装有脱脂棉的200 μL离心管接着穿过果柄韧皮部(引入部位见图3(a)),石蜡密封穿出孔,离心管内加200 μL浓度1 mg mL⁻¹的羧基荧光素酯(5,6-carboxyfluorescein diacetate bioreactive, CFDA)丙酮溶液,离心管盖扎孔,管身用锡箔纸包好. 处理24 h后采摘果实,冰盒带回实验室,徒手切片激光共聚焦扫描显微镜(confocal laser scanning microscopy, CLSM)观察.

为了清楚标记木质部的位置,将已经引入CFDA的果实采摘后去掉木质部以外部分,插入浓度1 mg mL⁻¹的德克萨斯红(dextran, Texas Red)中,处理30 min,徒手切片CLSM观察.

(viii) 组织徒手切片CLSM观察. 采摘CFDA处理后的果实,用冰盒带回实验室. 对果实主要维管束分别做横切和纵切徒手切片,切片迅速投入80%甘

油中防止荧光染料猝灭。将切片移到载玻片上,用盖玻片覆盖后CLSM观察CF和Texas-Red在无花果果实维管束内的转运情况(CF最适激发光波长490 nm,发射光波长515 nm; Texas-Red最适激发光波长595 nm,发射光波长615 nm)因此,CF采用488 nm激光激发,505~530 nm滤光片检测信号, Texas-Red采用568 nm激光激发,605~630 nm的滤光片检测信号。

2 结果

2.1 无花果果实生长曲线、可溶性糖和淀粉含量变化

无花果果实生长曲线呈双S形,从花芽分化结束到果实完全成熟可分为3个时期(图1(a), (b))。花芽分化结束后前30 d是第1个快速生长期,果实由小绿果快速膨大为绿果,果实大小增加了大约3倍,同时果实内部花序轴和小花都随之增大,但果实内还有空腔,小花没有上色。从花芽分化后30~65 d是第2个生长期缓慢生长期,无花果果实由绿果发育为始红果,果实大小增长缓慢,但果实逐渐退绿,果实内部小花发育迅速,开始由无色变为红色,果实内花序轴和小花逐渐增大,果实内空腔缩小几乎消失。花芽分化后65~85 d为第3个时期,即第2个快速生长期,果实由始红果发育为全红果,果实大小快速增加,从始红果到片红果10 d内果实增大了1.5倍,果实表面迅速开始上色,直到成熟时发育为紫红色果实,果实内部花序轴和小花随着果实膨大增大,果实内部再次出现空腔,小花顶部花柱和柱头由红色变为黄色。

随着无花果果实成熟,果实的可溶性糖和淀粉含量均发生一系列变化。通过对无花果果实发育过程可溶性糖和淀粉的测定,发现无花果果实可溶性糖含量以葡萄糖和果糖为主,蔗糖含量相对较低(图2)。随着无花果果实发育葡萄糖和果糖含量均呈现逐渐升高趋势,蔗糖一直维持在较低水平,淀粉含量呈逐渐降低的趋势。在小绿果、绿果、始红果时期葡萄糖和果糖含量缓慢升高,淀粉含量快速下降,始红果后的片红果和全红果葡萄糖和果糖含量快速升高,淀粉含量缓慢下降。

2.2 SE/CC复合体和周围薄壁细胞联系的超微结构观察

无花果是由花序轴顶端膨大并凹陷形成腔室,

腔室壁上着生许多单性小花组成的假果(图3(a)~(c))。发育中的无花果果实维管束由主要维管束和次要维管束组成(图3(b), (c))。主要维管束是无花果果实发育同化物运输的主要通道,为此本实验以无花果主要维管束为研究对象。超微结构透射电子显微镜观察发现在SE和CC之间存在大量胞间连丝(表1,图4(a), (b), (e)), SE/CC复合体和PCs之间,只有CC和PCs之间在小绿果、绿果、始红果时期有胞间连丝存在(表1,图4(a), (c), (e)),而在整个无花果果实发育过程中SE和PCs之间始终没有胞间连丝出现(表1,图4(a), (e))。PCs之间在无花果整个发育过程中存在大量胞间连丝(表1,图4(a), (d), (f), (g)),表明在韧皮部后运输过程中同化物通过共质体途径进行。

2.3 始熟期前CF从韧皮部卸出,始熟期后CF均被限制在韧皮部中

为了进一步验证无花果果实韧皮部同化物运输途径,将CFDA从果柄引入韧皮部,观察CF分布。CFDA是6(5)羧基荧光素(carboxyfluorescein, CF)的脂类形式,可以跨膜运输但没有荧光,在细胞内被分解为不能跨膜运输但能通过胞间连丝且有荧光的CF,从而通过CF分布确定韧皮部和PCs之间的同化物运输途径^[10,22]。CF引入无花果果柄后,大约需要2~3 h能到达果实,在引入24 h后,采摘果实徒手切片CLSM观察韧皮部及其周围荧光分布情况。结果表明,在小绿果(图5(a), (a'), (b), (b'))、绿果、(图5(c), (c'), (d), (d'))始红果(图5(e), (e'), (f), (f'))时期CF均从韧皮部卸出到周围组织中。在始熟期后的片红果(图5(g), (g'), (h), (h'))、全红果(图5(i), (i'), (j), (j'))时期横切面和纵切面CF均被严格限制在韧皮部内没有卸出。说明在始熟期前韧皮部和周围薄壁细胞存在共质体联系,同化物通过胞间连丝运输。在始熟期后韧皮部和周围薄壁细胞存在共质体隔离,同化物主要通过质外体途径运输。

3 讨论

3.1 始熟期伴随着韧皮部卸载路径从共质体向质外体的转换

无花果果实由缓慢生长期到第2次快速增长期之间红色箭头所指的始红果时期为无花果果实成熟的转折期(图1(a)),从这一时期开始果实淀粉含量降低,

图 1 无花果果实生长发育曲线及果实发育外观图。(a) 无花果果实纵横径变化图及采样时间(黑色箭头)。果实发育分 3 个时期, 时期 I 为第 1 个快速生长期; 时期 II 为缓慢生长期; 时期 III 为第 2 个快速生长期/成熟期。红色箭头所指第 2 期和第 3 期之间为始熟期; (b) 无花果外观和内部发育变化图

Figure 1 Seasonal growth curve and external fig fruit development view. (a) A schema showing the developmental stages of fig and sampling time (black arrow). There are three fruit development stages. Stage I, the first rapid growth phase; Stage II, the lag phase of growth; Stage III, the second rapid growth phase/ripening phase. The red arrow denotes the transition from Stage II to Stage III, onset of ripening; (b) The exterior and interior changes during fig fruit development

图 2 无花果果实可溶性糖和淀粉含量变化

Figure 2 The change of soluble sugars and starch during fig fruit development

果实可溶性糖含量快速升高, 果实迅速膨大, 果实表面快速上色, 果实迅速成熟, 因此这一时期为无花果

果实的始熟期, 是调控无花果果实从生长到成熟的重要转折期。

无花果果实超微结构透射电子显微镜观察发现在始熟期前CC/PCs之间存在胞间连丝(图4(c), 表1), 果实韧皮部和周围薄壁细胞之间存在共质体联系, 同化物能够通过共质体途径从韧皮部运输到周围薄壁细胞中。始熟期后CC/PCs之间没有发现胞间连丝(图4(e), 表1), 存在共质体隔离, 同化物运输主要通过质外体途径。

无花果果实始熟期后胞间连丝消失这一观察结果同葡萄^[13]和枣^[14]不同, 由共质体联系向共质体隔离转换时葡萄果实胞间连丝密度没有变化但是胞间连丝出现堵塞现象通透性下降, 枣果实胞间连丝数量出现明显减少, 并且也出现了堵塞的现象。无花果果实始熟期后胞间连丝消失这一现象可能是由于从这一时期开始果实迅速膨大, 对胞间连丝产生了稀

图 3 无花果果实组织结构和维管束分布图. (a) 完整无花果果实, 果柄处为CFDA引入处; (b) 无花果染色果实横切面图, 显示了维管束的分布情况, 以及采样部位; (c) 图(b)采样部位无花果果实横切面解剖图. 果实由花序轴和其上着生的花组成; (d) 无花果果实主要维管束及其周围果肉薄壁细胞横切面徒手切片图. 无花果主要维管束为双韧维管束

Figure 3 Structure of fig fruit and the distribution of the vascular bundles. (a) An intact fig fruit, showing the loading site of CFDA into the pedicel; (b) transverse section of dye-infused fig fruit, showing the distribution of the vascular bundles and the sampling site; (c) transverse anatomical section of the close-up of boxed region in (b). The fig fruit composite formed of rachis and tiny flowers; (d) free hand transverse section of major vascular bundle and its surrounding parenchyma cells of fig fruit. The major vascular bundles are bicollateral

释效应导致未能观察到, 另一个原因可能是细胞快速膨大, 细胞间间隙增大出现空腔, 胞间连丝被撕裂破坏而消失, 在烟草叶片源库转换过程中也出现了同样的现象^[23].

在活细胞水平上, 利用荧光示踪技术对各个发育时期的无花果果实韧皮部卸载路径进行研究, CLSM观察发现在始熟期前果实发育的第Ⅰ期和第Ⅱ期, 荧光物质均从韧皮部卸出(图5(a)~(f), (a')~(f')). 在始熟期后果实发育的第Ⅲ期, 荧光物质被限制在韧皮部内(图5(g)~(j), (g')~(j')). 结果进一步证实了始熟期是韧皮部卸载路径发生转变的转折期. 上述结果为无花果果实发育始熟期伴随共质体和质外体卸载途径的转变提供了明确的证据.

3.2 韧皮部卸载路径转换的可能机制和意义

植物库器官韧皮部同化物卸载途径分共质体和质外体^[21], 共质体途径是被动运输, 主要通过细胞间胞间连丝的溶液集流和扩散作用运输同化物, 具有较高的运输能力和较小的运输阻力^[4], 同化物经过

共质体卸载时是阻力最小的卸载方式, 不需要消耗能量. 质外体途径同化物直接跨膜运输, 可以通过蔗糖转运蛋白跨膜运输^[24], 或细胞壁蔗糖酶水解蔗糖为已糖然后通过 H^+ 已糖同向运输器跨膜运输^[25], 质外体卸载途径需要载体的参与, 需要消耗能量.

许多研究证明, 果实库器官韧皮部卸载途径形式多样, 如苹果^[11]、梨(*Pyrus spp.*)^[26]、黄瓜^[17]、草莓^[15]果实在整个发育过程中韧皮部均为质外体卸载. 还有的是两种卸载方式交替进行, 例如, 枣果实在发育前期和后期为质外体卸载, 而发育中期为共质体卸载方式^[14]. 无花果卸载方式同番茄^[16]、葡萄^[13]相一致, 均是始熟期前淀粉积累期韧皮部采用共质体途径, 在始熟期后已糖积累期韧皮部卸载采用质外体途径, 并且这3种果实均不积累蔗糖, 果实内可溶性糖主要为葡萄糖和果糖.

无花果果实韧皮部后运输始终是共质体运输而韧皮部运输形式却在始熟期后由共质体运输转变为质外体运输方式. 发生共质体隔离的原因可能是由于随着无花果果实成熟, 果实内的可溶性糖含量在

图 4 无花果果实发育过程中主要维管束筛管伴胞复合体及其周围薄壁细胞超微结构图。所有样品均为横切。(a) 全红果实时初期韧皮部主要维管束横切面图, 显示了SE-CC复合体及其周围PCs, 箭头所指为胞间连丝; (b) 小绿果时期SE-CC复合体及其之间的胞间连丝; (c) 大绿果CC/PC之间的胞间连丝图; (d) 大绿果PC/PC之间的胞间连丝; (e) 全红果SE/CC之间有胞间连丝, SE/PP和CC/PP之间没有胞间连丝; (f) 片红果PC/PC之间胞间连丝; (g) 全红果PC/PC之间胞间连丝。SE, 筛管; CC, 伴胞; PC, 薄壁细胞; Pd, 胞间连丝; G, 高尔基体

Figure 4 The ultrastructure of the sieve element-companion cell (SE-CC) complex and its surrounding parenchyma cells in the major vascular bundles of developing fig fruit. All sections were cut transversely. (a) A transverse section of the phloem in the major bundle in full red stage, showing the structure of SE-CC complex and its surrounding PCs, arrows indicate plasmodesmata; (b) plasmodesmata present between SE and CC in small green stage; (c) plasmodesmata between CC and PC in big green stage; (d) plasmodesmata between PCs in big green stage; (e) plasmodesmata between SC and CC in full red stage, note there was no plasmodesmata between SC and PC, CC and PC; (f) plasmodesmata between PCs in partial red stage; (g) plasmodesmata between PC and PC in full red stage. SE, sieve element; CC, companion cell; PC, parenchyma cell; Pd, plasmodesmata; G, golgi apparatus

表 1 无花果果实发育过程中韧皮部不同细胞类型间胞间连丝密度^{a)}
Table 1 Plasmodesmal densities between different cells in the phloem of developing fig fruit^{a)}

果实发育时期	SE/CC	SE/PC	CC/PC	PC/PC
小绿	0.76±0.30	0	0.82±0.13	0.77±0.14
大绿	0.59±0.15	0	1.04±0.56	0.76±0.16
始红	0.42±0.05	0	0.52±0.07	0.62±0.16
片红	0.35±0.05	0	0	0.54±0.10
全红	0.40±0.09	0	0	0.95±0.39

a) 单位: 横切面细胞界面平均长度上的胞间连丝数量(No. μm^{-1}). 每个值是均值细胞界面, 30个重复。SE: 筛分子; CC: 伴胞; PC: 薄壁细胞

进入始熟期后迅速升高, 可溶性糖主要积累在薄壁细胞内, 薄壁细胞间形成共质体联系有利于同化物的快速运输, 但薄壁细胞和筛分子伴胞复合体间如果同化物卸载主要通过集流扩散作用的共质体途径, 那么薄壁细胞内到筛管伴胞复合体的逆浓度梯度会导致同化物回流^[4,27], 同化物无法卸载。因此果实成熟期可溶性糖大量积累时韧皮部的共质体隔离防止糖分的回流, 促进果实同化物的积累。这种机制明显不同于正在生长的马铃薯块茎^[10]、根^[7,8]、叶^[9]等不大量积累可溶性糖的营养库。

图 5 无花果果实发育过程中CF卸载CLSM观察图。CF从果柄引入无花果果实后 2~3 h 到达果实(图 3(a))。采处理 24 h 的果实,徒手切片观察果实中部主要维管束(图 3(b)~(d))。图(g), (h)中的红色区域是被Texas Red dextran荧光探针标记的木质部。(a), (a'), (b), (b')样品为小绿果。(c), (c'), (d), (d')样品为大绿果。(e), (e'), (f), (f')样品为始红果。(g), (g'), (h), (h')样品为片红果。(i), (i'), (j), (j')样品为全红果。(a)~(j)为荧光图。(a')~(j')为明场图。(a), (a'), (c), (c'), (e), (e'), (g), (g'), (i), (i')为横切图;(b), (b'), (d), (d'), (f), (f'), (h), (h'), (j), (j')为纵切图。始熟期前CF从韧皮部卸载到周围薄壁细胞(a)~(f), (a')~(f'), 始熟期后CF被限制在韧皮部(g)~(j), (g')~(j')

Figure 5 CLSM imaging of CF unloading during fig fruit development. CF reached the fruit flesh phloem 2~3 h after loading at the pedicel (Figure 3(a)). The treated fruit was sampled 24 h after CF loading and the hand-sections were prepared from major vascular bundle zones in the central area (Figure 3(b)~(d)). The red areas in (g) and (h) indicate the xylem zone labeled by Texas Red detran. (a), (a') (b), (b') Small green fruit. (c), (c') (d), (d') Big green fruit. (e), (e') (f), (f') Initial red fruit. (g), (g') (h), (h') Partial red fruit. (i), (i') (j), (j') Full red fruit. (a)~(j) Fluore scence images. (a')~(j') Bright-fieldimages. (a), (a'), (c), (c'), (e), (e'), (g), (g'), (i), (i') Transverse sections. (b), (b'), (d), (d'), (f), (f'), (h), (h'), (j), (j') Longitudinal sections. Before onset of ripening CF was shown to be released from phloem and spread in PCs (a)~(f), (a')~(f'). After onset of ripening CF was restricted to the phloem strands along the phloem pathway in the vascular bundle (g)~(j), (g')~(j')

4 结论

无花果果实在始熟期前果实可溶性糖含量低,果实韧皮部卸载途径为共质体途径;果实在始熟期

后可溶性糖含量迅速升高,韧皮部卸载路径转换为质外体途径。始熟期是无花果果实韧皮部卸载路径的转折期。无花果果实韧皮部后运输为共质体途径。

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A shift of phloem unloading from symplasmic to apoplastic pathway during fig fruit development

LI ChunLi¹, HOU BingZhu², ZHANG XiaoYan³, LI Jiang¹ & SHEN YuanYue²

¹ College of Forest Science and Horticulture, Xinjiang Agricultural University, Urumqi 830052, China;

² Beijing Key Laboratory of New Technology in Agricultural Application, College of Plant Science and Technology, Beijing University of Agriculture, Beijing 102206, China;

³ College of Biology, China Agricultural University, Beijing 100094, China

To uncover the phloem unloading pathway of fig fruit, five-stage fruit of Masui Dauphine fig (*Ficus carica* L., *Moraceae*) were used to research, including small green, big green, initial red, partial red, and full red, at about 15, 30, 65, 70 and 85 d after flower bud differentiation. A combination of electron microscopy and phloem-mobile symplasmic tracer carboxyfluorescein (CF), the phloem unloading pathway was studied in the developmental fruit, meanwhile, fruit size and color, as well as soluble sugar and starch contents were also analysis.

The growth of fig fruit follows a typical sigmoid growth curve with distinct three developmental stages: stage I, the first rapid growth phase; stage II, the lag phase; stage III, the second rapid growth phase, namely, turning and ripening stages; the transition from stage II to stage III represents the onset of ripening. During stage I and stage II, fruit soluble sugar contents is lower, while the onset of the fruit ripening, a rapid increase in both fruit volume and soluble sugar contents with main glucose and fructose was observed. The contents of starch were decreasing rapidly before the onset of ripening, then after, decreased slowly. Electron microscopy observations showed that the sieve element-companion cell (SE-CC) complex of the vascular bundles is apparently symplasmically connected through plasmodesmata with surrounding parenchyma cells (PCs) during the stage I and stage II, but a transition occurred from symplastic to apoplastic after the onset of the fruit ripening. Interestingly, an abundance of plasmodesmata was always observed between parenchyma cells during the fruit development. Imaging of carboxyfluorescein movement showed that the dye is released from the functional phloem strands before the onset of ripening, whereas the CF was confined to the phloem strands after the onset of ripening, considering that a transient and critical developmental checkpoint is present around the onset of ripening about 1–2 days, and the apoplastic unloading pathway contributes to high accumulation of soluble sugars.

Before the onset of the fruit ripening, the soluble sugar content is low concentration, which is consistent with the sugar accumulation with a symplasmic pathway. After the onset of the fruit ripening, a rapid increase in soluble sugar content is resulted from sugar accumulation by apoplastic pathway. The phloem-unloaded higher soluble sugars through apoplastic pathway, as a result, may result in a considerable turgor pressure in PCs; manifestly, such high turgor pressure, if the SE-CC complex were symplasmically connected with surrounding PCs via plasmodesmal channels, the phloem-unloaded sugars may be released again to sieve tubes, not maintaining higher soluble sugars in PCs. In addition, an abundance of plasmodesmata between PCs also contribute to high accumulation of soluble sugars in the sink tissues, which promote fruit ripening.

The results obtained from the present study indicate that the phloem unloading pathway switches from a symplasmic pathway before onset of ripening to an apoplastic pathway in the late stage; subsequently, post-phloem transport of sugar follows a symplasmic pathway in fig fruit.

fig fruit, phloem unloading, ultrastructure, carboxyfluorescein imaging, symplasmic, apoplastic

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