砧/穗间交流的物质及其嫁接愈合效应

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摘要 植物嫁接愈合受砧/穗自身和外部环境等多种因素的调节和控制。在嫁接伤口愈合期间, 砧木与接穗的相互作用不仅体现在明显的组织学特征的变化, 还涉及了复杂的生理生化和分子调控机制。砧木和接穗愈合经历隔离层的形成、愈伤组织的发生、分化以及维管组织的再分化四个步骤, 在此过程中砧/穗细胞、组织或器官之间相互影响, 频繁发生物质交流, 如植物激素、蔗糖、核酸和蛋白质等物质通过胞间连丝发生短距离运输或是经韧皮部进行长距离运输, 从而开始形成砧/穗之间的识别、接纳、包容效应, 最终完成愈合过程, 并对嫁接苗后续生长过程起到重要影响。该文就当前植物嫁接过程中砧/穗愈合的组织学特征、砧/穗间交流物质的属性及其对砧/穗愈合进程的影响进行综述, 为植物嫁接繁殖的生产实践提供基础理论指导。

关键词 砧木; 接穗; 亲和性; 物质运输途径; 嫁接愈合影响因子

The Exchange Substance of Rootstock and Scion and Its Grafting Healing Effect

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Abstract Plant graft healing is regulated by rootstock/scion themselves and the external environmental factors. The interaction between rootstock and scion is not only present histological characteristic changes, but also involves complex physiological, biochemical and molecular regulatory mechanisms during the graft healing. Rootstock and scion have completed healing by undergoing four steps of isolation layer formation, callus formation, differentiation and vascular tissue redifferentiation. Essentially, the cells, tissues or organs of rootstock and scion interact with each other and frequently exchange substances, such as plant hormones, sugar, nucleic acids and proteins by short-distance transporting through plasmodesmata, as well as by long-distances transporting over phloem in the process of grafting healing. Thus, the recognition, acceptance and inclusion effect between the rootstock and scion is established, and the graft healing process is finished finally. It also plays an important role in the subsequent growth process of grafted seedlings. In this paper, the histological characteristic, exchanges substances property and the factors which are affected the healing process have been reviewed, and it will provide the basic theoretical guidance for the production practice of plant graft propagation.

Keywords rootstock; scion; compatibility; transportation routes of substances; factors affecting graft healing

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嫁接是进行大规模营养繁殖的方法之一[1], 最初用于改善作物的农艺性状, 现在广泛应用于良种选育和园艺作物栽培等领域, 并创造出了丰厚的经济效益。将一个植物体的枝或芽等部位嫁接到另一种植物枝、干或根上时, 枝或芽可以来自同一个体(自体嫁接), 或来自两个不同个体(异体嫁接)[2], 因此, 接穗与砧木愈合过程中会频繁发生物质交流, 小分子(蔗糖、激素等)、大分子(核酸、蛋白质等)物质可在胞间连丝中发生短距离运输[3-4], 或是经韧皮部进行长距离运输[5]。在植物嫁接过程中, 砧/穗愈合不仅受到外界等多种因素的调节和控制, 如温度、光照、湿度等影响着砧/穗嫁接成功与否[6], 砧/穗间交流的物质也会对砧/穗愈合进程产生巨大影响。该文综述了植物嫁接愈合的组织学特征、砧/穗之间物质运输及影响嫁接愈合的因素等方面的研究进展, 解析了砧/穗间物质运输途径, 分析了糖类、激素等因素对嫁接愈合进程的影响, 展望了嫁接繁殖的研究趋势, 旨在为农林生产实践提供理论指导。

1 砧/穗愈合的组织学特征

砧木和接穗是否可以嫁接成功的关键之一是砧木与接穗的细胞组织之间能愈合。在组织学水平上, 嫁接愈合需经历隔离层的形成、愈伤组织的发生、分化以及维管组织的再分化四个步骤[7-8]。隔离层的形成, 使砧木或接穗内部组织与外界分开, 所产生的物质促进伤口的愈合和抵抗病原物的入侵[8]。在隔离层形成的同时, 结合处周围韧皮部、木质部薄壁细胞等发生脱分化形成愈伤组织。随着愈伤组织细胞快速分裂, 隔离层逐渐变薄并被突破, 在砧/穗的结合处充满愈伤组织, 填充砧/穗间的空隙[9], 愈伤组织的薄壁细胞相互抱合、连接形成愈伤组织桥, 实现砧木为接穗提供营养和水分的功能[10]。其后, 部分愈伤组织细胞分化为维管形成层, 与砧木和接穗的形成层连接形成完整的形成层环, 随后形成层环向外形成次生韧皮部, 向内形成木质部和次生木质部, 将砧/穗连为一体[11]。值得注意的是, 砧/穗连接处组织的分化具有不对称性, 一般韧皮部的分化早于木质部的分化[12]。对于嫁接失败的嫁接组合来说, 砧/穗间薄壁细胞胞间连丝不充分偶合[13], 组织连接具有滞后性, 出现维管束连接不规则、后期嫁接部位组织羸弱的现象, 最终导致嫁接失败[14]。

2 砧/穗间物质交流与运输

砧/穗之间的结合涉及复杂的形态生理过程。嫁接后砧/穗细胞之间相互接触, 引发物质在砧/穗间交流与运输。HERTLE等[15]发现在砧/穗愈伤组织增殖期间, 质体脱分化形成小的、变形虫状的可运动的细胞器, 同时, 新的连接细胞间通过细胞壁局部解体形成连接孔, 变形虫状细胞器通过连接孔进入新细胞中。这一工作揭示了细胞器从一个细胞向另一个细胞转移的路径, 为基因组的水平转移提供了例证。事实上, 砧/穗愈合过程中物质交流与信息转导频繁, 不仅包括了核酸、蛋白质等大分子物质(表1)[16], 而且包括了糖类[4]、生物碱[17]、激素[18]等小分子物质(表2)。

2.1 大分子物质的交流与运输

在真核生物进化过程中, 基因的水平转移(horizontal gene transfer, HGT)是非常普遍的现象[19]。早在2009年, STEGEMANN等[3]构建两种转基因烟草(*Nicotiana tabacum*)株系, 将此两种株系嫁接后进行抗性筛选和荧光信号分析, 发现两种株系嫁接后的烟草细胞质间发生了基因交流, 认为质体基因组能够在紧密相邻的细胞间转移。为进一步证明质体基因组在植物细胞间转移后的遗传稳定性, 将荧光标记的烟草与光烟草(*Nicotiana glauca*)和本氏烟草(*Nicotiana benthamiana*)进行嫁接, 结果表明种间遗传信息的传递, 在很大程度上局限于叶绿体DNA的移动而不涉及核基因组片段的移动, 并且质体基因组的移动毫无方向性[20]。FUENTES等[21]的工作给出了不同的结论, 认为嫁接后的植株具有砧木和接穗植物总和的染色体数目, 说明烟草属植物间核基因组可以相互转移。利用由于携带波缘烟草(*Nicotiana undulata*)线粒体基因组而导致雄性不育的烟草作为接穗, 林烟草(*Nicotiana sylvestris*)雄性可育株系为砧木, 嫁接后发现烟草的育性恢复, 说明线粒体基因组也可以在砧/穗间转运[22]。但是, DNA的移动似乎仅限于细胞间短距离的移动, 只通过胞间连丝进行胞间转移或交流[23]。

mRNA主要运输方式是长距离运输, 高等植物韧皮部是RNA的主要运输通道[24]。数以千种的mRNA可以在拟南芥(*Arabidopsis thaliana*)[25]、葡萄(*Vitis girdiana*)[26]、黄瓜(*Cucumis sativus*)[27]等植物的砧/穗间进行长距离运输。利用组学和荧光标记技术, 研究者可以鉴别和检测砧/穗间运输的mRNA

表1 砧/穗间大分子物质的交流与运输

Table 1 Exchange and transportation of macromolecular substance between rootstock and scion

接穗 Scion	转运方向 Transfer direction	砧木 Rootstock	物质种类 Type of substance	文献 Reference
Nuc-kan:yfp	—	Pt-spec:gfp	cpDNA	[3]
Nicotiana tabacum	—	*Nicotiana glauca*; *Nicotiana benthamiana*	cpDNA	[20]
Nicotiana tabacum	—	*Nicotiana sylvestris*	mtDNA	[22]
Nicotiana glauca	—	*Nicotiana tabacum*	DNA	[21]
Nicotiana tabacum	—	*Nicotiana glauca*	DNA	[21]
Solanum lycopersicum	←	Transgenic *Solanum lycopersicum*	ToFT	[42]
Cucurbita moschata	←	*Cucurbita maxima*	Cm-FTL1/2	[45]
Cucurbita maxima	←	*Cucumis melo*	CmmLec17	[48]
Solanum lycopersicu	→	*Solanum lycopersicum*	SLCyp1	[49]
Transgenic *Arabidopsis thaliana*	→	*Arabidopsis thaliana*	FNR	[46]
Vitis vinifera	←	Transgenic *Vitis vinifera*	PGIP	[50]
Cucumis sativus	←	*Cucurbita maxima*	PP1/2	[51]
Pyrus bretschneideri	↔	*Pyrus betulifolia*	*PbWoxT1* mRNA	[33]
Cucumis sativus	←	*Cucurbita maxima*	*CmNACP* mRNA	[27]
Malus domestica	↔	*Malus xiaojinensis*	*GAI* mRNA	[32]
Solanum lycopersicum	←	*Nicotiana sylvestris*	*NsCET1* mRNA	[52]
Solanum tuberosum	→	*Solanum tuberosum*	*StBEL5*/11/29 mRNA	[29-30]
Arabidopsis thaliana	↔	*Arabidopsis thaliana*	*PS* mRNA	[53]
Malus domestica	→	*Malus xiaojinensis*	*MdOPT3* mRNA	[31]
Prunus mahaleb ‘Gisela 6’	←	Transgenic *Prunus mahaleb* ‘Gisela 6’	siRNA	[37]
Vitis vinifera	↔	*Vitis riparia*	siRNA	[2]
Transgenic *Arabidopsis thaliana*	→	*Arabidopsis thaliana*	miR399	[38-39]
Transgenic *Solanum tuberosum*	→	*Solanum tuberosum*	miR156	[40]
Transgenic *Glycine max*	←	*Glycine max*	miR172a	[41]

—: 在砧/穗结合部发生短距离运输, 方向不确定。箭头表示物质在砧/穗间的转运方向。

—: the short-distance transport occurs at the junction of the rootstock and scion, and there is no direction exactly. The arrows indicate the direction of transport of the substance between the rootstock and the scion.

表2 砧/穗间小分子物质之间的交流与运输

Table 2 Exchange and transportation of small molecules substance between rootstock and scion

接穗 Scion	转运方向 Transfer direction	砧木 Rootstock	物质种类 Type of substance	文献 Reference
Solanum lycopersicum	←	*Nicotiana tabacum*	Nicotine	[17]
Solanum lycopersicum	→	*Solanum lycopersicum*	Abscisic acid	[18]
Arabidopsis thaliana	←	*Arabidopsis thaliana*	Gibberellin	[59]
Transgenic *Arabidopsis thaliana*	→	*Arabidopsis thaliana*	Gibberellin	[60]
Nicotiana tobaccum	←	Transgenic *Nicotiana tobaccum*	Auxin	[58]
Nicotiana tabacum ‘Yunyan 87’	←	*Nicotiana tabacum* ‘Wufeng 2’	K^+	[55]

箭头表示物质在砧/穗间的转运方向。

The arrows indicate the direction of transport of the substance between the rootstock and the scion.

种类以及运输的方向, 发现不同种类的mRNA运输方向各不相同。在农作物土豆(*Solanum tuberosum*)中, *StBEL5/11/29* mRNA与*POTH1* mRNA均由接穗运输至砧木[28-30], 而笋瓜(*Cucurbita maxima*)的*CmNACP* mRNA则由砧木转运至黄瓜接穗[27]。木本植物苹果(*Malus domestica*)的*MdOPT3* mRNA可由

接穗转运至砧木[31], 但苹果的*GAI* mRNA[32]和杜梨(*Pyrus betulifolia*)的*PbWoxT1* mRNA[33]均被证实具有双向运输的特性。

植物小分子RNA(sRNA)是长度在21~24 nt的非编码小分子RNA, 可分为miRNA和siRNA两大类[34], 参与基因转录后调控。移动的sRNA在受体细胞中直接进行表观遗传修饰, 对植物产生持久的影响[35]。LEWSEY等[36]发现24 nt sRNA可以由接穗转移到砧木中去, 并介导受体细胞的DNA甲基化。在'Gisela 6'甜樱桃(*Prunus mahaleb* 'Gisela 6')中, PNRSV-hpRNA衍生的siRNA可由砧木转移到非转基因接穗中, 并通过试验证实这些siRNA有助于增强接穗对病毒抵抗力[37]。同样, miRNA也可以在砧/穗间交流和运输。比如, 拟南芥miR399可以从接穗运输至砧木[38-39]; 在农作物中, miR156可以从土豆接穗运输至砧木[40]; 而miR172a被证实在大豆(*Glycine max*)中可以由砧木运输至接穗[41]。

蛋白质也可以通过植物韧皮部进行长距离运输[42]。事实上, 植物体大分子物质转运的认知始于蛋白质分子的长途运输。早在1994年, 以笋瓜和黑籽南瓜(*Cucurbita ficifolia*)为砧木、黄瓜为接穗进行异体嫁接试验, 并利用聚丙烯凝胶电泳技术检测到接穗内具有砧木所含的蛋白质成分, 推测蛋白质通过嫁接部位的韧皮部运输至接穗中[43], 后来GOLECKI等[44]进一步研究认为砧/穗之间蛋白质的运输在葫芦科植物中是普遍的现象。开花的笋瓜砧木可以将开花信号传递到南瓜(*Cucurbita moschata*)接穗中, 因此在南瓜接穗的韧皮部检测到Cm-FTL1和Cm-FTL2蛋白的大量存在[45]。WU等[42]发现当野生型番茄(*Solanum lycopersicum*)接穗嫁接到35S:*ToFT*和35S:*AtFT*转基因番茄砧木上时, 可在野生型番茄接穗中检测到ToFT-GFP和AtFT-GFP融合蛋白, 而且这些蛋白通过韧皮部向野生型接穗转运促进幼年番茄接穗成花。将质体转运肽标记GFP后获得的转基因拟南芥接穗, 嫁接到非转基因拟南芥砧木上10天后, 在根分生组织附近发现荧光信号[46]。至于韧皮部中所鉴定到一些行使辅助运输功能的蛋白质, 则被认为是大分子物质进行长距离运输的重要组成部分, 这类蛋白与RNA分子结合, 可以增加胞间连丝的通透极限, 在一定程度上亦辅助RNA的选择性运输[47]。在杜梨嫁接试验中, 也发现多聚嘧啶序列结合蛋白PbPTB3可以与*PbWoxT1* mRNA相互作用, 并介导mRNA通过韧皮部进行长距离的运输[33]。

2.2 小分子物质的交流与运输

嫁接愈合过程也涉及到多种小分子物质的运输。离子在砧/穗间运输是小分子物质运输的主要方式之一。嫁接试验显示, Na^+、K^+由根向地上部进行选择性运输, 从而影响地上部分的Na^+和K^+的含量[54], 并且有证据表明砧木和接穗可以互相调控离子吸收的数量。胡玮[55]以不同基因型的烟草作为砧木, 分析比较K^+向叶片的运输状况, 发现不同砧木对K^+向叶片运输的影响有显著差异。在不同品种西瓜(*Citrullus lanatus*)嫁接试验中, "勇士"作为接穗的嫁接苗, 其根系K^+含量显著高于"早佳8424"作为接穗的嫁接苗, 可见"勇士"作为接穗可显著提高砧木对K^+的吸收能力, 这是接穗对砧木的反调控现象[56]。前期研究资料显示, 作为植物小分子代谢物的生物碱也会在砧/穗间运输。以烟草与番茄的嫁接试验为例, 在嫁接后番茄果实的烟碱水平显著增加, 这是因为烟碱通过运输作用在番茄果实中大量累积[17]。可溶性糖是植物生长发育主要能源物质, 在砧/穗愈合过程中发挥巨大作用。在甜樱桃(*Prunus avium*)中, 砧木会影响可溶性糖的流动, 其经过运输后在嫁接结合处积累[57]。ZHAI等[58]发现嫁接在含有生长素合成基因*iaaM*(编码农杆菌的色氨酸2-单加氧酶)砧木上的烟草接穗中, 内源性生长素水平升高以及生长素响应基因*IAA8*和*DAO*(dioxygenase of auxin oxidation)表达量增加, 意味着生长素可由砧木运输至接穗。同样, 脱落酸(ABA)和赤霉素(GA)也可以在砧/穗间运输。以ABA缺陷型的番茄突变体为砧木, 将野生型番茄作为接穗, 结果发现砧木根部中有大量的ABA积累[18]。将野生型拟南芥和GA缺陷突变体进行嫁接时, 野生型的砧木产生的GA, 可以通过嫁接部位移动至接穗中, 并进一步转化为具有生物活性的GA[59]。GA也可以由接穗向砧木运输, 且接穗的GA信号影响砧木木质部的分化[60]。

3 影响砧/穗嫁接愈合的物质组分

在嫁接过程中, 砧/穗间物质交流频繁、种类丰富, 在一定程度上影响着砧/穗的愈合以及嫁接苗的生长状况。关于嫁接苗形态与生长状态变化的研究资料甚少, 研究结果也集中于模式植物中[21,42], 相关

调控机制的研究结论不明确, 但嫁接愈合进程中对砧/穗间交流的物质(主要包括糖类、酚类、激素、核酸、蛋白质等)影响研究资料较多。

3.1 糖类及酚类物质

一般地, 糖类物质的过量积累会影响砧/穗间愈伤组织的形成。FREY等[4]观察到在番茄不亲和的嫁接组合中, 可溶性糖和淀粉会出现不平衡, 接穗中的可溶性糖含量会明显高于砧木中的含量, 而亲和性较强的嫁接组合在嫁接几天后韧皮部就会恢复运输功能, 砧/穗间糖类物质不平衡的现象会渐渐消失。以枳(*Citrus trifoliate*)为砧木、柚(*Citrus maxima*)为接穗进行嫁接, HE等[61]发现可溶性糖的大量积累会作为一种调节信号, 诱发植物生长的停止以及诱导代谢过程的发生, 从而导致韧皮部损伤, 并伴有细胞壁变形和增厚。当然, 也有研究表明可溶性糖和淀粉的积累并不是直接影响愈合的关键因素。譬如, LOUPIT等[62]认为虽然葡萄(*Vitis riparia*)嫁接结合处存在大量的糖分积累, 但是其成活率并未受到显著影响。

酚类化合物对嫁接愈合的调控作用主要是通过阻碍生长素的运输完成的。ERREA[63]发现酚类物质很容易被过氧化物酶氧化产生醌类物质, 阻碍生长素运输, 导致砧木和接穗连接受阻, 后期研究也发现李属(*Prunus*)不亲和组合间的愈伤组织中存在大量的酚类物质[13]。不同种类的酚类物质, 对植物嫁接愈合影响的效果也不同。利用青钱柳(*Cyclocarya paliurus*)为接穗, 薄壳山核桃(*Carya illinoinensis*)为砧木, 李娜等[64]发现嫁接处单宁含量较高, 导致隔离层扩增, 嫁接成活率较低。葡萄嫁接砧/穗组合中愈伤组织发育程度越高, 黄烷醇浓度越低[65]。同样, 在黄瓜为接穗、南瓜为砧木的嫁接组合中, 不亲和嫁接组合的结合处木质素含量高度累积, 阻碍接穗和砧木的连接[66]。另外, 二苯乙烯代谢物浓度与嫁接成功之间也具有很强的相关性。在不亲和的葡萄属(*Vitis*)植物组合(UB/RSB1)中, 二苯乙烯二聚体在嫁接处聚集, 最终导致嫁接成活率降低[62]。

3.2 植物激素

激素对嫁接植物的愈伤组织分化具有促进或者抑制的作用, 从而影响着嫁接愈合状况[67]。表3显示了参与嫁接愈合过程激素的种类、相关基因表达及其调控效果。

在不同品系油茶(*Camellia oleifera*)的芽苗砧嫁接试验中, 发现‘18号’品种砧木茎段创伤后吲哚乙酸(IAA)、反式玉米素(TZR)、玉米素(ZT)、水杨酸(SA)都高于‘53号’砧木品种, 而这些激素含量上升有益于愈伤组织形成[71]。但是, 茉莉酸(JA)会影响愈伤组织的形成[67]。值得注意的是, MATSUOKA等[70]利用拟南芥嫁接试验验证了JA对于细胞增殖过程并不是必需的。在植物砧/穗愈合阶段, IAA在维管组织形成中起着核心作用, 外源性IAA应用于未分化的组织, 可以促进维管组织的形成[67]。同样, 细胞分裂素(CTK)被证明可以在受伤的茎中诱导维管组织分化[72]。CUI等[73]发现番茄会在嫁接后3~12 h时产生生长素和细胞分裂素效应, 促进木质部和韧皮部的分化。此外, 乙烯(ETH)可以促进细胞的增殖, 加速愈伤组织的形成, 因为使用乙烯生物合成抑制剂会延缓野生型烟草自体嫁接的愈合进程[58]。激素类别之间还具有协同作用, ETH和JA可以共同协作促进*ANAC071*的表达, 而*ANAC071*可以控制髓细胞增殖[74]。IAA、GA和CTK也具有协同作用, 能刺激细胞分化, 促进维管束的形成及木质部和韧皮部的重新连接[75]。

3.3 核酸与蛋白质

核酸、蛋白质等大分子物质也会对嫁接愈合过程产生影响。运用蛋白质双向电泳结合质谱技术, 冯金玲等[76]在油茶芽苗砧嫁接接口处, 分析获得了40个差异蛋白, 分析9个蛋白可能会与嫁接愈合有

表3 植物激素及其相关基因的作用

Table 3 The roles of plant hormones and related genes involved in grafting

植物激素 Plant hormone	相关基因 Related gene	调控效果 Regulating effect	参考文献 Reference
Auxin	*ANAC071*, *RAP2.6L*	Promote callus connection	[68]
Auxin	*XTH19*, *XTH20*	Encodes proteins involved in cell proliferation during healing	[58]
ETH (ethylene)	*ACS1*, *ERF5*	Affects callus formation	[58]
Cytokinin	*CYCD3;1*, *WIND1*	Promotes callus formation	[67,69]
JA (jasmonic acid)	*DAD1*, *AOS*, *JAZ10*	Induction of *RAP2.6L* expression at the grafting site	[67-68,70]

关, 推测嫁接一方面刺激嫁接口基因转录和蛋白质翻译; 另一方面刺激部分酶活性, 提高细胞抗性, 促进油茶芽苗砧嫁接愈合。在葡萄嫁接3天至28天后, 有37个涉及细胞壁合成、降解的基因会在嫁接界面处上调表达[77]。同样, miR156、miR159、miR160、miR172、miR390b和miR482可能参与山核桃(*Carya cathayensis*)愈合组织的形成[78]。MO等[79]进一步研究发现, miRS26促进了愈伤组织的形成, 而miR156、miR160、miR164、miR166和miRS10则影响着砧/穗维管组织的连接。显然, 由于核酸和蛋白质的存在, 嫁接口处信号转导、能量代谢、物质合成过程处于活跃的状态, 有利于促进嫁接愈合进程。

4 展望

嫁接相连意味着接通来自不同个体的两个植物器官构成了一种很独特的共生关系, 使二者成为一个共同体。因此, 砧/穗间交流的物质通过影响接口处组织分化从而影响嫁接成活率, 并进一步影响嫁接苗的质量。事实上, 不仅糖类物质、小分子代谢物以及植物激素可以在砧/穗间进行交流, 而且DNA、RNA、蛋白质等大分子物质也可以在砧/穗间进行长距离或短距离的双向或单向运输(图1)。这些交流的物质直接或间接影响砧/穗之间组织愈合的状态, 决定着嫁接组合的亲和程度, 对植物形态改变以及生长繁育起到关键的调控作用。目前, 关于嫁接愈合效应调控机制的认知还不够深入, 所以未来砧/穗间交流运输的物质及其作用研究应着力于以下几个方面。(1) 砧/穗之间物质交流频繁, 这些物质对砧/穗愈合的作用, 尤其是对愈合后嫁接苗生长的影响研究资料较少, 因此深入探讨植物嫁接过程中交流物质的功能, 特别是针对砧/穗愈合组织学特征变化影响物质种类的甄别, 对于植物异体嫁接愈合效应的阐明意义巨大。(2) 激素是影响嫁接成活的直观生理指标, 涉及相关嫁接愈合进程中基因的表达、代谢产物的形成等诸多环节, 利用外源激素来提升嫁接成活率也是生产实践中易于操作的技术方法。在分析内源激素影响的基础上, 探索不同外源激素的作用效果不失为提高嫁接成活率重要技术措施。(3) 鉴于植物糖类及酚类物质对砧/穗愈合影响作用, 因此有效抑制妨碍嫁接愈合的代谢产物的形成, 探索克服砧/穗间不亲和性的方法, 是快速繁育经济农林植物的必要保障。(4) 由于不同砧木

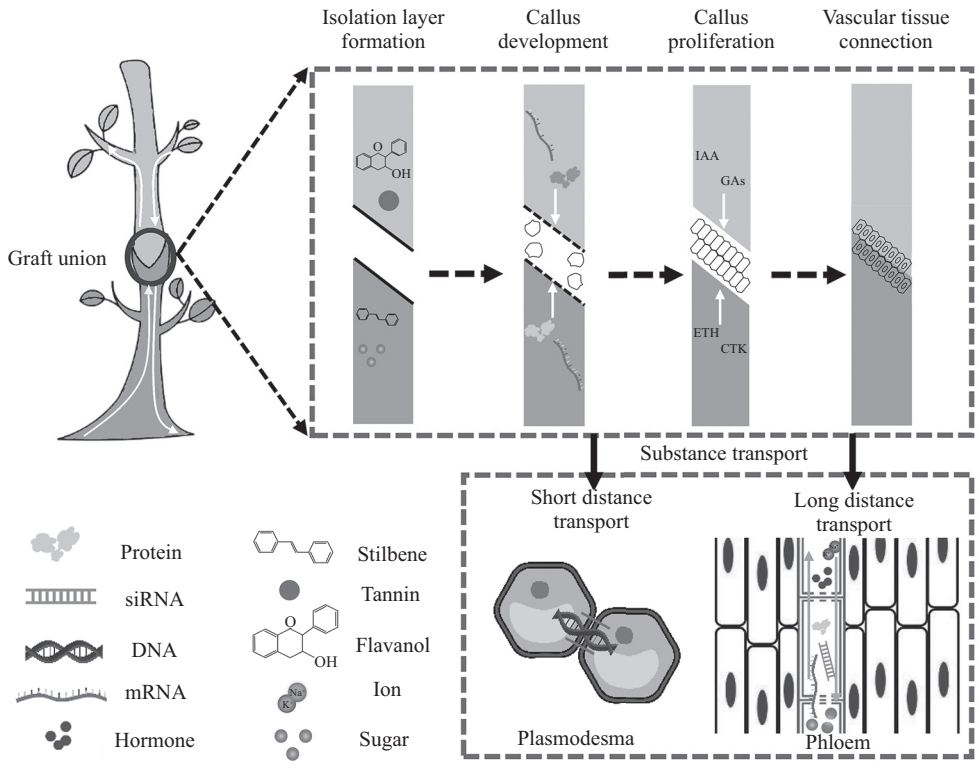

图1 嫁接愈合过程中物质运输及其愈合效应示意图

Fig.1 Schematic diagram of substance transport and its effects during graft healing

的遗传特性可赋予植物地上部分的独特性状, 所以筛选不同的遗传个体作为砧木来改良植物性状是未来生产实践的重要需求, 因此探索促进砧/穗间物质交流的方法, 是利用嫁接技术创制优势性状种质资源的重要研究方向。总体来说, 解析砧/穗间物质交流状况是深入研究嫁接愈合机理的有效途径之一, 而探索砧/穗间交流的物质对嫁接成功与否的影响意义巨大。因此, 利用转录组学、基因组学、蛋白质组学、代谢组学的方法, 综合探究砧/穗之间的相互作用机制, 快速筛选亲和且砧/穗优势性状明显的嫁接组合, 充分挖掘嫁接愈合的影响因子, 创制促进砧/穗快速愈合的方法, 可为农林生产实践提供技术支撑。

参考文献 (References)

[1] GOLDSCHMIDT E E. Plant grafting: new mechanisms, evolutionary implications [J]. Front Plant Sci, 2014, 5: 727.

[2] RUBIO B, STAMMITTI L, COOKSON S J, et al. Small RNA populations reflect the complex dialogue established between heterograft partners in grapevine [J]. Hortic Res, 2022, 9: uhab067.

[3] STEGEMANN S, BOCK R. Exchange of genetic material between cells in plant tissue grafts [J]. Science, 2009, 324(5927): 649-51.

[4] FREY C, ALVAREZ R, ENCINA A, et al. Tomato graft union failure is associated with alterations in tissue development and the onset of cell wall defense responses [J]. Agronomy, 2021, 11(6): 1197.

[5] ZHAO D, ZHONG G Y, SONG G Q. Transfer of endogenous small RNAs between branches of scions and rootstocks in grafted sweet cherry trees [J]. PLoS One, 2020, 15(7): e236376.

[6] 陈瑶, 孙李勇, 赵雨萌, 等.中国玉兰嫁接繁殖技术研究进展[J]. 世界林业研究(CHEN Y, SUN L Y, ZHAO Y M, et al. Research Advances in magnolia grafting propagation techniques in china [J]. World Fore Res), 2021, 34(4): 84-8.

[7] KUROTANI K I, NOTAGUCHI M. Cell-to-cell connection in plant grafting-molecular insights into symplasmic reconstruction [J]. Plant Cell Physiol, 2021, 62(9): 1362-71.

[8] 祁利潘, 李越, 王磊, 等. 马铃薯与枸杞嫁接愈合过程的解剖学观察[J]. 园艺学报(QI L P, LI Y, WANG L. Anatomical observation on the graft union between potato and wolfberry [J]. Acta Hortic Sin), 2022, 49(4): 868-74.

[9] MELNYK C W. Plant grafting: insights into tissue regeneration [J]. Regeneration, 2017, 4(1): 3-14.

[10] 李越. 马铃薯与枸杞嫁接愈合过程解剖学观察及属间有性杂交初探[D]. 张家口: 河北北方学院, 2020.

[11] 王春梅, 沈珊, 王红, 等.枫杨砧木与核桃嫁接接合部愈合过程的解剖学研究[J]. 中国南方果树(WANG C M, SHEN S, WANG H, et al. Anatomical study on the healing process of the grafted joint between maple poplar rootstock and walnut [J]. China S Fruit), 2022, 51(1):118-23.

[12] XU C, WU F, GUO J, et al. Transcriptomic analysis and physiological characteristics of exogenous naphthylacetic acid application to regulate the healing process of oriental melon grafted onto squash [J]. Peer J, 2022, 10: e13980.

[13] PINA A, ERREA P, MARTENS H J. Graft union formation and cell-to-cell communication via plasmodesmata in compatible and incompatible stem unions of *Prunus* spp. [J]. Sci Hortic, 2012, 143: 144-50.

[14] DOGRA K, KIRAN K, KUMAR R, et al. Graft-incompatibility in horticultural crops [J]. Int J Curr Microbiol Appl Sci, 2018, 7: 1805-20.

[15] HERTLE A P, HABERL B, BOCK R. Horizontal genome transfer by cell-to-cell travel of whole organelles [J]. Sci Adv, 2021, 7(1): 203-46.

[16] THOMAS H R, FRANK M H. Connecting the pieces: uncovering the molecular basis for long-distance communication through plant grafting [J]. New Phytol, 2019, 223(2): 582-89.

[17] YASINOK A E, SAHIN F I, EYIDOGAN F, et al. Grafting tomato plant on tobacco plant and its effect on tomato plant yield and nicotine content [J]. J Sci Food Agr, 2009, 89(7): 1122-28.

[18] MANZI M, LADO J, RODRIGO M J, et al. Root ABA accumulation in long-term water-stressed plants is sustained by hormone transport from aerial organs [J]. Plant Cell Physiol, 2015, 56(12): 2457-66.

[19] PRASAD A, CHIROM O, PRASAD M. Horizontal gene transfer and the evolution of land plants [J]. Trends Plant Sci, 2022, 27(12): 1203-05.

[20] STEGEMANN S, KEUTHE M, GREINER S, et al. Horizontal transfer of chloroplast genomes between plant species [J]. Proc Natl Acad Sci USA, 2012, 109(7): 2434-38.

[21] FUENTES I, STEGEMANN S, GOLCZYK H, et al. Horizontal genome transfer as an asexual path to the formation of new species [J]. Nature, 2014, 511(7508): 232-35.

[22] GURDON C, SVAB Z, FENG Y, et al. Cell-to-cell movement of mitochondria in plants [J]. Proc Natl Acad Sci USA, 2016, 113(12): 3395-400.

[23] DONG D, SHI Y N, MOU Z M, et al. Grafting: a potential method to reveal the differential accumulation mechanism of secondary metabolites [J]. Hortic Res, 2022, 9: uhac050.

[24] LI W, CHEN S, LIU Y, et al. Long-distance transport RNAs between rootstocks and scions and graft hybridization [J]. Planta, 2022, 255(5): 96.

[25] THIEME C J, ROJAS-TRIANA M, STECYK E, et al. Endogenous *Arabidopsis* messenger RNAs transported to distant tissues [J]. Nat Plants, 2015, 1(4): 15025.

[26] YANG Y, MAO L, JITTAYASOTHORN Y, et al. Messenger RNA exchange between scions and rootstocks in grafted grapevines [J]. BMC Plant Biol, 2015, 15: 251.

[27] RUIZ-MEDRANO R, XOCONOSTLE-CAZARES B, LUCAS W J. Phloem long-distance transport of *CmNACP* mRNA: implications for supracellular regulation in plants [J]. Development, 1999, 126(20): 4405-19.

[28] MAHAJAN A, BHOGALE S, KANG I H, et al. The mRNA of a Knotted1-like transcription factor of potato is phloem mobile [J]. BMC Plant Biol, 2012, 79(6): 595-608.

[29] BANERJEE A K, CHATTERJEE M, YU Y, et al. Dynamics of a mobile RNA of potato involved in a long-distance signaling pathway [J]. Plant Cell, 2006, 18(12): 3443-57.

[30] GHATE T H, SHARMA P, KONDHARE K R, et al. The mobile

RNAs, *StBEL11* and *StBEL29*, suppress growth of tubers in potato [J]. Plant Mol Biol, 2017, 93(6): 563-78.

[31] LÜ X, SUN Y, HAO P, et al. RBP differentiation contributes to selective transmissibility of OPT3 mRNAs [J]. Plant Physiol, 2021, 187(3): 1587-604.

[32] XU H, ZHANG W, LI M, et al. *Gibberellic acid insensitive* mRNA transport in both directions between stock and scion in *Malus* [J]. Tree Genet Genomes, 2010, 6(6): 1013-9.

[33] DUAN X, ZHANG W, HUANG J, et al. *PbWoxT1* mRNA from pear (*Pyrus betulaefolia*) undergoes long-distance transport assisted by a polypyrimidine tract binding protein [J]. New Phytol, 2016, 210(2): 511-24.

[34] BERGER M M J, GALLUSCI P, TEYSSIER E. Roles of epigenetic mechanisms in grafting and possible applications. in: advances in botanical research [M]. London: Academic Press, 2018.

[35] MOLNAR A, MELNYK C W, BASSETT A, et al. Small silencing RNAs in plants are mobile and direct epigenetic modification in recipient cells [J]. Science, 2010, 328(5980): 872-75.

[36] LEWSEY M G, HARDCASTLE T J, MELNYK C W, et al. Mobile small RNAs regulate genome-wide DNA methylation [J]. Proc Natl Acad Sci USA, 2016, 113(6): E801-E10.

[37] ZHAO D, SONG G Q. Rootstock-to-scion transfer of transgene-derived small interfering RNAs and their effect on virus resistance in nontransgenic sweet cherry [J]. Plant Biotechnol J, 2014, 12(9): 1319-28.

[38] LIN S, CHIANG S, LIN W, et al. Regulatory network of microRNA399 and *PHO2* by systemic signaling [J]. Plant Physiol, 2008, 147(2): 732-46.

[39] PANT B D, BUHTZ A, KEHR J, et al. MicroRNA399 is a long-distance signal for the regulation of plant phosphate homeostasis [J]. Plant J, 2008, 53(5): 731-8.

[40] BHOGALE S, MAHAJAN A S, Natarajan B, et al. MicroRNA156: a potential graft-transmissible microRNA that modulates plant architecture and tuberization in *Solanum tuberosum* ssp. *Andigena* [J]. Plant Physiol, 2014, 164(2): 1011-27.

[41] PAN W J, TAO J J, CHENG T, et al. Soybean miR172a improves salt tolerance and can function as a long-distance signal [J]. Mol Plant, 2016, 9(9): 1337-40.

[42] WU Y, MA Y, WANG M, et al. Mobility of FLOWERING LOCUS T protein as a systemic signal in trifoliate orange and its low accumulation in grafted juvenile scions [J]. Hortic Res, 2022, 9: uhac056.

[43] TIEDEMANN R, CARSTENS-BEHRENS U. Influence of grafting on the phloem protein patterns in Cucurbitaceae. I. additional phloem exudate proteins in *Cucumis sativus* grafted on two *Cucurbita* species [J]. J Plant Physiol, 1994, 143(2): 189-94.

[44] GOLECKI B, SCHULZ A, CARSTENS-BEHRENS U, et al. Evidence for graft transmission of structural phloem proteins or their precursors in heterografts of Cucurbitaceae [J]. Planta, 1998, 206(4): 630-40.

[45] LIN M, BELANGER H, LEE Y, et al. FLOWERING LOCUS T protein may act as the long-distance florigenic signal in the Cucurbits [J]. Plant Cell, 2007, 19(5): 1488-506.

[46] PAULTRE D, GUSTIN M P, MOLNAR A, et al. Lost in transit: long-distance trafficking and phloem unloading of protein signals in *Arabidopsis* homografts [J]. Plant Cell, 2016, 28(9): 2016-025.

[47] KEHR J, BUHTZ A T. Long distance transport and movement of RNA through the phloem [J]. J Exp Bot, 2008, 59(1): 85-92.

[48] GOMEZ G, TORRES H, PALLAS V. Identification of translocatable RNA-binding phloem proteins from melon, potential components of the long-distance RNA transport system [J]. Plant J, 2005, 41(1): 107-16.

[49] SPIEGELMAN Z, HAM B K, ZHANG Z, et al. A tomato phloem-mobile protein regulates the shoot-to-root ratio by mediating the auxin response in distant organs [J]. Plant J, 2015, 83(5): 853-63.

[50] AGUERO C B, URATSU S L, GREVE C, et al. Evaluation of tolerance to Pierce's disease and Botrytis in transgenic plants of *Vitis vinifera* L. expressing the pear PGIP gene [J]. Mol Plant Pathol, 2005, 6(1): 43-51.

[51] GOLECKI B, SCHULZ A, THOMPSON G A. Translocation of structural P proteins in the phloem [J]. Plant Cell, 1999, 11(1): 127-140.

[52] HUANG N, LUO K, YU T. Mobility of antiflorigen and PEBP mRNAs in tomato-tobacco heterografts [J]. Plant Physiol, 2018, 178(2): 783-94.

[53] ZHANG H, YU P, ZHAO J, et al. Expression of tomato prosystemin gene in *Arabidopsis* reveals systemic translocation of its mRNA and confers necrotrophic fungal resistance [J]. New Phytol, 2018, 217(2): 799-812.

[54] 白丽萍, 何雨, 宋宇, 等.茄子砧木Na^+、K^+含量、$S_{K,Na}$运输与耐盐性关系研究[J]. 植物生理学报(BAI L P, HE Y, SONG Y, et al. Study on relationship between Na^+、K^+ content and $S_{K,NA}$ of transport of eggplant rootstocks and salt-tolerance [J]. Plant Physiol J), 2014, 50(11): 1645-650.

[55] 胡玮. 嫁接对烤烟钾素吸收利用的影响及其机理研究[D]. 重庆: 西南大学, 2019.

[56] 焦妍妍. 嫁接西瓜接穗对砧木钾吸收的反馈调控机理[D]. 武汉: 华中农业大学, 2017.

[57] OLMSTEAD M A, LANG N S, LANG G A. Carbohydrate profiles in the graft union of young sweet cherry trees grown on dwarfing and vigorous rootstocks [J]. Sci Hortic, 2010, 124(1): 78-82.

[58] ZHAI L, WANG X, TANG D, et al. Molecular and physiological characterization of the effects of auxin-enriched rootstock on grafting [J]. Hortic Res, 2021, 8(1): 1-13.

[59] REGNAULT T, DAVIERE J M, WILD M, et al. The gibberellin precursor GA12 acts as a long-distance growth signal in *Arabidopsis* [J]. Nat Plants, 2015, 1: 15073.

[60] RAGNI L, NIEMINEN K, PACHECO-VILLALOBOS D, et al. Mobile gibberellin directly stimulates *Arabidopsis* hypocotyl xylem expansion [J]. Plant Cell, 2011, 23(4): 1322-36.

[61] HE W, XIE R, WANG Y, et al. Comparative transcriptomic analysis on compatible/incompatible grafts in *citrus* [J]. Hortic Res, 2022, 9: uhab072.

[62] LOUPIT G, VALLS FONAYET J, PRIGENT S, et al. Identifying early metabolite markers of successful graft union formation in grapevine [J]. Hortic Res, 2022, 9: uhab070.

[63] ERREA P. Implications of phenolic compounds in graft incompatibility in fruit tree species [J]. Sci Hortic, 1998, 74(3): 195-205.

[64] 李娜, 朱培林, 丰采, 等. 青钱柳嫁接愈合过程中砧穗生理特性及其与亲和性的关系[J]. 南京林业大学学报(LI N, ZHU P

L, FENG C, et al. Variations in physiological characteristics of rootstock-scion and its relationship to graft compatibility during the grafting union process of *Cyclocarya paliurus* [J]. J Nanjing For Univ), 2021, 45(1): 13-20.

[65] PRODHOMME D, VALLS F J, HEVIN C, et al. Metabolite profiling during graft union formation reveals the reprogramming of primary metabolism and the induction of stilbene synthesis at the graft interface in grapevine [J]. BMC Plant Biol, 2019, 19(1): 599.

[66] MIAO L, LI S, BAI L, et al. Effect of grafting methods on physiological change of graft union formation in cucumber grafted onto bottle gourd rootstock [J]. Sci Hortic, 2019, 244: 249-56.

[67] NANDA AK, MELNYK CW. The role of plant hormones during grafting [J]. J Plant Res, 2018, 131(1): 49-58.

[68] PITAKSARINGKARN W, ISHIGURO S, ASAHINA M, et al. *ARF6* and *ARF8* contribute to tissue reunion in incised *Arabidopsis* inflorescence stems [J]. Plant Biotechnol, 2014, 31(1): 49-53.

[69] IKEUCHI M, IWASE A, RYMEN B, et al. Wounding triggers callus formation via dynamic hormonal and transcriptional changes [J]. Plant Physiol, 2017, 175(3): 1158-74.

[70] MATSUOKA K, YANAGI R, YUMOTO E, et al. *RAP2.6L* and jasmonic acid-responsive genes are expressed upon *Arabidopsis* hypocotyl grafting but are not needed for cell proliferation related to healing [J]. Plant Mol Biol, 2018, 96(6): 531-42.

[71] 龙伟, 姚小华, 吕乐燕. 基于芽苗砧嫁接油茶砧穗创伤后内源激素动态变化分析[J]. 植物研究(LONG W, YAO S, LÜ L Y. Dynamic changes of endogenous hormones in rootstocks and scions within nurse seedling graft in *Camellia oleifera* under wound [J]. Bull Bot Res), 2021,41(2): 232-42.

[72] ALONI B, COHEN R, KARNI L, et al. Hormonal signaling in rootstock-scion interactions [J]. Sci Hortic, 2010, 127(2): 119-26.

[73] CUI Q, XIE L, DONG C, et al. Stage-specific events in tomato graft formation and the regulatory effects of auxin and cytokinin [J]. Plant Sci, 2021, 304: 110803.

[74] ASAHINA M, AZUMA K, PITAKSARINGKARN W, et al. Spatially selective hormonal control of *RAP2.6L* and *ANAC071* transcription factors involved in tissue reunion in *Arabidopsis* [J]. Proc Natl Acad Sci USA, 2011, 108(38): 16128-32.

[75] 张捷, 艾迪, 孟景祥, 等. 植物嫁接砧木与接穗互作机制研究进展[J]. 西北农林科技大学学报(ZHANG J, AI D, MENG J X, et al. Research progress on interactive mechanism between rootstock and scion after plant grafting [J]. J Northwest Sci Tech Univ), 2022, 50(5): 139-45.

[76] 冯金玲, 杨志坚, 陈辉. 油茶芽苗砧嫁接口不同发育时期差异蛋白质分析[J]. 应用生态学报(FENG J L, YANG Z J, CHEN H. Differential protein analysis of different developmental periods at the grafting mouth of oil tea shoot rootstock [J]. Chi J Appl Ecol), 2012, 23(8): 2055-61.

[77] COOKSON S J, CLEMENTE M M, HEVIN C, et al. Graft union formation in grapevine induces transcriptional changes related to cell wall modification, wounding, hormone signaling, and secondary metabolism [J]. J Exp Bot, 2013, 64(10): 2997-3008.

[78] SIMA X, JIANG B, FANG J, et al. Identification by deep sequencing and profiling of conserved and novel hickory microRNAs involved in the graft process [J]. Plant Biotechnol Rep, 2015, 9(3): 115-24.

[79] MO Z, FENG G, SU W, et al. Identification of miRNAs associated with graft union development in pecan [*Carya illinoinensis* (Wangenh.) K. Koch] [J]. Forests, 2018, 9(8): 472.