综 述 Reviews

间歇浸没式液体培养技术及其在莓类果实植物微扩繁中的应用

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摘要: 建立高效种苗组织培养快速繁殖技术对于保障莓类果实植物优质高产、满足日益增长的市场需求具有重要意义。本文综述间歇浸没式液体培养技术的发展及其在植物微扩繁中的应用, 分析和评价该技术在草莓、树莓、蓝莓等几种重要莓类植物繁殖中的研究现状, 并展望了其在莓类植物研究应用领域的发展前景。

关键词: 间歇浸没; 液体培养; 莓类植物; 植物微扩繁

莓类果实植物为双子叶多年生被子植物, 全球范围内最重要的莓类果实主要集中在蔷薇科的草莓属和悬钩子属, 以及杜鹃花科的越橘属, 具体包括草莓(*Fragaria* × *ananassa* Duch.)、树莓(*Rubus idaeus* L.)、黑莓(*Rubus* spp.)和蓝莓(*Vaccinium* spp.)等(朱传根等2015; Debnath 2011)。莓类果实具有酸甜可口、香气浓郁、营养丰富和颜色多样等诸多优点, 日常食用对人类健康大有益处(Kowalska和Olejnik 2016), 所以近几年深受人们的喜爱, 具有良好的销售市场。植物微扩繁(plant micropropagation)是提高莓类果实植物种苗繁殖速度、缩短育苗周期并获得优质种苗的首选方法。针对培养基类型、植物生长调节剂配比、外植体类型和培养条件等已经开展了较多的研究工作(Debnath 2007; 朱丽君等2014; 吴光洪等2016; 阳翠等2016), 为莓类植物的大规模扩繁提供了研究基础和技术参考。

液体培养基的可流动性使其易于实现自动化控制, 同时能为培养物提供均衡的营养物质, 可以在不更换培养容器的情况下实现培养基的更新, 且能配置较大的培养容器。在早期, 人们将培养物完全浸没在液体培养基中, 往往导致氧气不足及植株超度含水现象(hyperhydricity, 又称玻璃化)的发生(Watt 2012), 而间歇浸没式液体培养技术的出现逐渐克服了这些局限(赵望锋和王力华2007)。该技术通过间歇浸没式培养系统(temporary immersion system, TIS)对繁殖体进行培养, 提高了小植株质量且实现了大规模繁殖的目的。国外在20世纪80年代就开始了该项技术的研究, 目前技术已经成熟, 大规模用于农、林业和食品等产业中(Meyer等2009; Peña-Ramírez等2010; Snyman等2011a; 许亚良和张家明2013)。国内相关研究起步较晚, 近些年来发展较快, 利用该技术扩繁的植物种类逐渐增多(杨柳等2010a, 2010b, 2011; 许亚良和张家明2013; 张转转等2014; 石琨和郑彩霞2015; 高美萍等2016), 推动了国内一些经济作物优质种苗的大规模生产。目前, 国内对莓类果实植物的研究大多采用传统的固体/半固体培养方式, 该方式限制了高效的半自动化控制技术的应用, 使莓类果实植物优质种苗的栽培推广缓慢, 建立莓类植物高效种苗组织培养快速繁殖技术对于保障莓类果实植物优质高产、满足日益增长的市场需求具有重要意义。本文概述了间歇浸没式液体培养技术的发展, 评估TIS培养在植物微扩繁中的应用, 分析和评价该技术在草莓、树莓、蓝莓等几种重要莓类植物繁殖中的研究现状, 指出了其在其他相关领域的辅助应用趋势及在莓类植物上的发展前景。

1 间歇浸没式液体培养技术的发展

TIS也被称为间歇浸没式生物反应器, 主要利用液体培养基的间歇浸没来培养植物细胞、组织或器官。早期出现的TIS被称为automated plant culture system (APCS) (Tisserat和Vandercook 1985), 它主要由4部分组成: 植物培养箱、玻璃营养液蓄池、叶轮泵和硅胶运输管道。该系统由微型计算机控制, 在无菌环境下实现液体培养基的定期补充和排出, 减少了繁琐的继代更新的手工操作。

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之后Aitken-Christie和Davies等研究人员在1988年对APCS进行了改进, 利用蠕动泵和定时器实现了液体培养基的自动化间歇更新(Berthouly和Etienne 2005)。Simonton等(1991)也设计了一套由计算机控制泵装置的液体间歇供应系统。上述几种培养系统的液体培养基浸没驱动力都为机械动力, 虽然减少了劳动力耗费, 但是受空间限制, 操作复杂, 污染问题比较严重(Simonton等1991; Etienne和Berthouly 2002; 赵望锋和王力华2007)。

Recipient for automated temporary immersion (RITA®) (Alvard等1993)是一套由上下两部分相连组成的TIS, 该培养容器的上室包含培养物, 下室含有液体培养基, 利用气压为推动力, 将液体培养基压入上室与培养物接触, 同时引起气体流动, 实现培养容器空气的更新, 空气经过气体过滤膜除菌(图1)。相继, twin flasks system (BIT®) (Escalona等1999), 即两个分开的容器(培养容器和营养液储存容器)组成的双瓶式TIS也被开发出来(图2)。近十几年, 研究人员根据实际需求设计或改造出许多类型的TIS, 有ebb and flood、immersion by bubbles (BIB®) (Paek等2005; Watt 2012)和Box-in-Bag (Ducos等2008)等。Stanly等(2010)根据RITA®的原理, 利用可重复使用的Nalgene®聚砜过滤系统改造了TIS容器; Yan等(2010)在研究中使用Plantima容器制成了类似RITA®的培养系统。郑彩霞等(2013)开发了一种可自动换液的双瓶间歇浸润式植物微扩繁器, 它既可实现自动更换营养液又可防止交叉污染, 提高了装置的自动化和灵活性。

目前, 已有几种类型的TIS成功应用于农林植物的微扩繁(Debnath 2009a; Jova等2011; Ashraf等2013), 实现了几种重要经济作物的大规模生产。其中, 双瓶式TIS (BIT®及各种变化的双瓶式系统)和RITA®应用较为广泛。在我国使用最广泛的是双瓶式TIS, 其培养容器的大小可按需改变, 构造简洁, 价格便宜。RITA®容器体积较小, 价格较贵, 可能限制了在发展中国家的推广应用(赵望峰和王力华2007)。

2 间歇浸没式液体培养扩繁植物的优缺点

2.1 繁殖的小植株质量好且增殖率高

培养物不是全程浸没在液体培养基中, 使其在间断液体浸没时可与气体接触, 避免长期浸没对植物生长和形态发生带来的不良影响。由于培

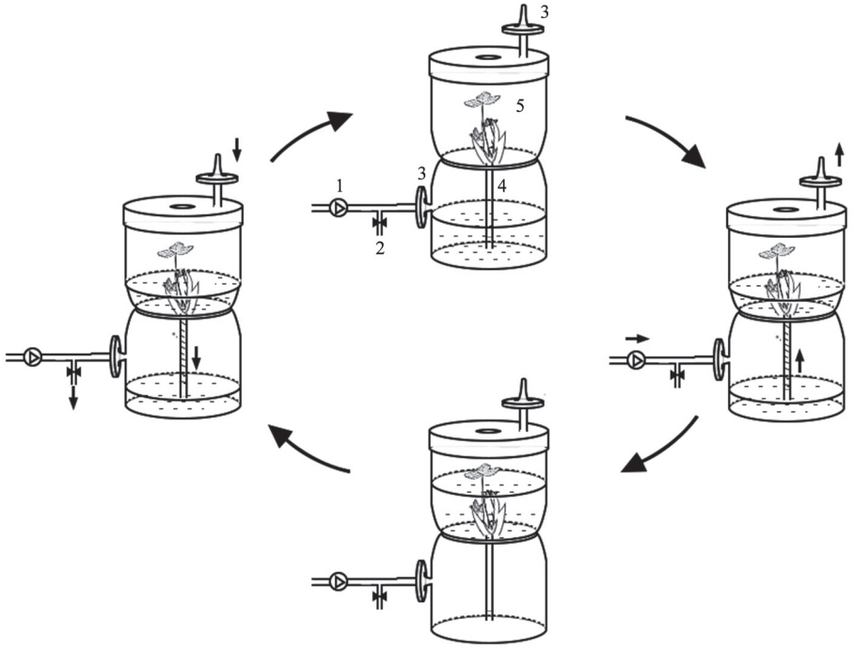

图1 RITA®培养系统示意图

Fig.1 Diagrammatic representation of RITA® system

参考Alvard等(1993)、Berthouly和Etienne (2005)文献修改。1: 气泵; 2: 电磁阀; 3: 滤菌器; 4: 连接管; 5: 植物培养室。

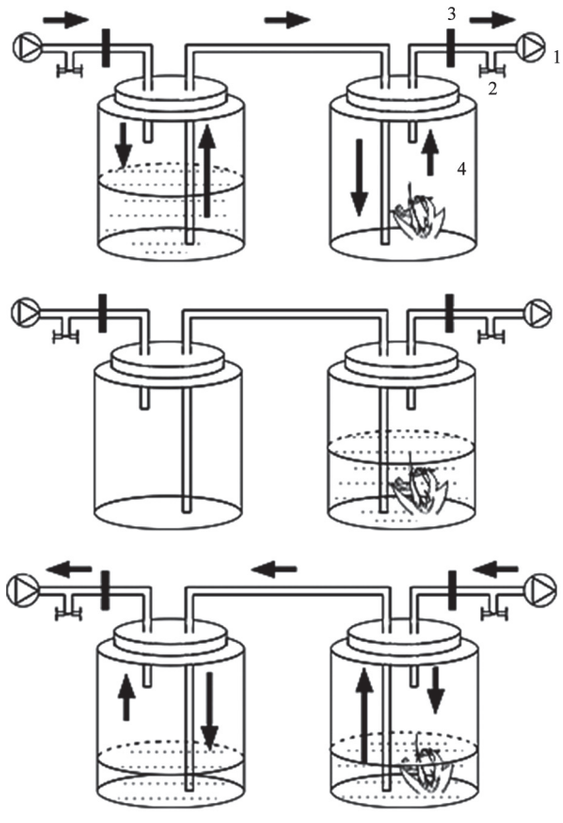

图2 BIT®培养系统示意图

Fig.2 Diagrammatic representation of BIT® system

参考Escalona等(1999)文献修改。1: 气泵; 2: 电磁阀; 3: 滤菌器; 4: 植物培养容器。

养容器内空气定时更新和植物对液体培养基的间歇接触, 其叶绿素含量、净光合速率、蒸腾速率等生理参数得到了改善(Jova等2011)。其次, 间歇浸没式液体培养可以提供充足的营养和氧气供应, 降低不利气体在培养环境中的积累, 例如, 可以比固体培养大大降低培养空间中CO_2和C_2H_4的浓度(Roels等2006), 从而保证了小植株的生长速度、增殖率和质量。利用TIS培养, 可使继代培养次数相对减少, 培养基易于及时更新, 使培养材料保持一致的培养环境和培养状态, 可明显提高扩繁苗的质量和增殖率, 提高植物扩繁产量(Roels等2005; 赵望锋和王力华2007; Watt 2012)。

2.2 小植株易于移栽驯化

不同的体外扩繁方法得到的小植株的生理状态不尽相同, TIS获得的植株移栽驯化阶段一般比传统固体和液体培养的植株更易存活(Etienne和Berthouly 2002)。在驯化阶段, 相比固体培养的植株, TIS培养的植株的光合速率较高, 在遮阴温室生长一段时间后其叶面积较大、鲜重和干重较高(Yang和Yeh 2008); 有些植株会展现出一种激活的抗氧化防御系统, 使它们能够应对瓶外或移栽环境带来的胁迫(Aragón等2010)。这表明TIS培养的小植株更易于驯化和适应移栽环境。

对移栽到田间的TIS再生植株进行生长调查发现, 同传统扩繁培养相比, 在田间生长初期, 植株在茎粗和株高等参数上有一些不同, 但这些差异随着栽培时间的延长逐渐消失(Lorenzo等2001)。通常, TIS生产的组培苗移栽大田后不会出现表型变异现象, 其遗传稳定性高(Snyman等2011b; Fki等2011), 这可能与使用TIS培养植物材料时, 所用植物生长调节剂的量一般不高, 培养周期相对较短有一定关系(Watt 2012)。

2.3 缺点及其对策

尽管间歇浸没式液体培养具有独特的优势, 但也存在一定的问题, 如易受微生物污染和出现超度含水苗等。

外植体(带节茎段、芽等)上常常携带内源性细菌或真菌, 这些微生物一旦暴露于液体培养基中便会迅速繁殖造成污染, 这是TIS扩繁某些植株过程中损失的主要原因(McAlister等2005; Watt等2006)。因此, 在大规模商业性苗木培养中, 此类事件会显著增加成本。目前虽然可用血清学技术、蛋白质和遗传分析等技术来处理内源性和潜在的污染, 但这一般需要应用专门的仪器并耗费较多的劳力(Watt 2012)。

常用的防止污染方法是对外植体进行消毒处理并同时在培养基中加入一定浓度的抗微生物物质, 这些物质有抗生素、杀菌剂(如: plant preservative mixture, PPM™; Vitrofural G-1)和纳米银(silver nanoparticles, AgNPs)等(McAlister等2005; González-Olmedo等2005; Luna等2008; Spinoso-Castillo等2017)。另外一种常用的有效方法是先在固体培养基上培养外植体, 筛选出无菌培养材料再进行TIS培养, 这种方法适合处理从田间采集的外植体(Mordocco等2009), 很多文献报道的研究策略都是利用此方法开展TIS研究, 以消除微生物污染和培养过程中的损失(杨柳等2010b; Niemenak等2013; 石琨和郑彩霞2015)。

培养材料超度含水是一种生理的紊乱现象, 严重时会导致培养材料坏死(Berthouly和Etienne 2005)。在TIS培养中, 虽然出现芽超度含水率通常比在传统液体培养中的低(Wawrosch等2005; Stanly等2010), 但是仍然不可避免出现玻璃化苗现象。有些植物的组培苗在固体培养时并未出现超度含水现象, 而在间歇浸没式液体培养时, 随浸没时间延长和浸没频繁提高, 会使50%的增殖芽出现超度含水症状(Shaik等2010)。因此, TIS中浸没的持续时间和频率是决定性的参数, 会影响培养物养分和水分的吸收, 影响培养材料的超度含水率。研究表明, 相对较高的浸没频率、较短的浸没时间或较长的间歇时间能刺激体细胞胚胎发生, 并降低甚至消除超度含水现象, 从而提高小植株的生产率(Teisson和Alvard 1995; Albarrán等2005; Gatica-Arias等2008)。

TIS中培养基的养分对培养材料的超度含水率影响也较显著。利用RITA®或者双瓶式系统微扩繁植株时, 在增加培养间歇时间的同时, 降低培养基养分水平, 会显著降低小植株的超度含水率(Snyman等2011b; Watt 2012)。

3 几种重要莓类果实植物的间歇浸没式液体培养

3.1 草莓

Hanhineva等(2005)使用含液体MS培养基[添加9 μmol·L^{-1}噻苯隆(thidiazuron, TDZ)和2.5 μmol·L^{-1}吲哚丁酸(indole-3-butyric acid, IBA)]的TIS (RITA®), 以5个草莓品种的叶片为外植体, 成功获得了不定芽, 研究结果表明, TIS培养的芽再生率[(70±8)%~(94±2)%]与固体培养的[(83±5)%~(92±3)%]相比无显著差异, 但是TIS培养所需的人力与时间不到固体培养的一半, 且继代培养时, 更换培养基等操作简单易行。证明该方法适于草莓不定芽再生和随后的继代培养。Debnath (2008)将TIS (RITA®)与固体培养相结合, 建立了草莓不定芽再生、增殖和生根的方法。将草莓叶片、萼片和叶柄先接种于固体培养基(添加2~4 μmol·L^{-1} TDZ)中培养一段时间, 再转入相同激素水平的TIS (浸没频率为每4 h浸没15 min)中培养, 获得丛生芽, 并用TIS对不定芽进行继代增殖。增殖后的丛生芽再转接入固体培养基中促使芽伸长和生根, 获得的组培苗驯化移栽大田后表型正常, 生长良好。该研究结果表明, TIS培养时会引起丛生芽超度含水, 而将这些超度含水芽转接到固体培养基(添加2~4 μmol·L^{-1}玉米素)中培养则能获得正常芽。该方法也提高了草莓的扩繁效率。

国内应用TIS进行草莓微扩繁的研究处于起步阶段, 间歇浸没式液体培养系统以双瓶式为主。张乔丽等(2014)利用‘红颜’组培苗为材料进行间歇浸没式增殖培养, 结果表明, TIS培养的增殖率约是传统培养的3倍; 这与张转转等(2014)的研究结果基本一致。柳燕等(2015)对固体与TIS两种组培方式下草莓扩繁效果及其获得的组培穴盘苗光合特性进行了比较分析, 得到TIS培养下草莓个体生物量和苗增殖系数都极显著优于固体培养的植株, 穴盘苗的光合速率、气孔导度和呼吸效率显著优于固体培养的苗, 胞间CO_2浓度两者差异不显著。

3.2 树莓

Debnath (2010)在成功建立了基于TIS的草莓高效扩繁技术后, 又开发出以叶片为外植体, 应用TIS结合固体培养扩繁3种北美红树莓的生产步骤。在增殖培养时使用了TDZ和6-苄氨基腺嘌呤(6-benzylaminopurine, 6-BA), 没有用生长素; 生根培养时培养基没有添加植物生长调节剂, 这可降低成本和减少出现体细胞无性系变异的可能性, 该扩繁树莓的步骤更简单且扩繁植株所需时间更短。Arencibia等(2013b)利用双瓶式TIS并控制其培养环境为高CO_2浓度(0.55 mL·L^{-1})、高光强(80 μmol·m^{-2}·s^{-1})及低蔗糖浓度(15 g·L^{-1})的方法扩繁树莓组培苗, 获得的植株在植株大小、总节数和驯化成活率上都具有优势。同时, 该研究也证明了利用间歇浸没式液体培养可以在培养过程中控制多种环境因素, 进而提高苗木的繁殖率和质量。

3.3 蓝莓

Debnath (2009b)对几种不同类型的矮丛蓝莓(*Vaccinium angustifolium*)首次进行了TIS扩繁技术的研究。他同样使用TIS结合固体培养的方法, 将固体培养获得的不定芽转接入TIS中进行增殖培养, 成功获得了丛生芽, 这为矮丛蓝莓或其他越橘属植物的大规模扩繁提供了参考。随后, Arencibia等(2013a)采用与树莓间歇浸没式液体培养相似的方法, 控制双瓶式TIS中的环境为富含CO_2、高光强和低蔗糖培养基的培养条件, 获得了相比蓝莓

固体培养更好的扩繁效果, 并且移栽入温室中的TIS蓝莓苗比传统培养的植株适应性更强, 生长速度更快。

4 小结与展望

间歇浸没式液体培养技术的发展和优势, 引领了崭新的半自动/自动方式的植物微扩繁技术(Watt 2012)。该技术优于传统固体和液体培养方式, 且其获得的植株在温室和田间表现良好, 已经被应用于多种植物的繁殖和育种以加快良种生产并节约成本。目前主要应用的间歇浸没式液体培养系统是双瓶式和RITA®。研究者在降低其培养中出现的不利因素上逐步采取了相应的对策, 取得了一定进展, 从而使该技术能更好发挥优势。随着间歇浸没式液体培养技术的成熟发展, 其在植物培养材料的代谢物质合成及分析(Niemenak等2008; Péres-Alonso等2009; Ashraf等2013)和细胞代谢途径调控(Ivanov等2012)等领域的辅助应用也被开发出来, 应用范围更广。

莓类果实因其较高的抗氧化性、抗癌等功效在世界范围内受到广泛关注, 发展前景广阔, 目前我国的莓类果实产业和相关研究正在蓬勃发展中, 市场对草莓、树莓和蓝莓等莓类植物的脱毒和高质量苗木的需求日益迫切。综上所述, 间歇浸没式液体培养技术在几种莓类植物上的微扩繁应用已经有了一定的基础, 但在培养条件上, 不同植物及其品种间存在差异, 尚需根据研究和应用目标进一步的优化, 以促进莓类果实相关产业的发展。

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The technology of temporary immersion in liquid culture and its application in micropropagation of berry plants

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Abstract: The establishment of a high efficiency micropropagation system for plant seedlings is critically important for high quality and yield production of berry plants to meet the increasing market demand. The development of temporary immersion technology in liquid culture and its application in plant micropropagation was reviewed. The research of the temporary immersion culture for propagation in several important berry plants such as strawberry, raspberry and blueberry was discussed and evaluated, and the development prospect of this technique in the research and application fields of berry plants was projected.

Key words: temporary immersion; liquid culture; berry plants; plant micropropagation

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