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牡丹组织培养技术研究进展*

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摘 要: 牡丹是我国特有的观赏、药用和油用植物,高效的组织培养技术是加速其育种和繁殖的重要基础。牡丹组织培养技术历经约半个世纪的研究,主要进展包括以下 3 个方面: 1) 器官组织培养再生: 牡丹器官组织培养再生主要包括器官发生、体细胞胚发生和无菌短枝扦插这 3 条途径。(1) 牡丹器官发生途径包括愈伤组织和分生结节培养,牡丹的各种繁殖器官和营养器官皆可作为外植体诱导得到愈伤组织,但愈伤组织进一步分化器官难度较大,且尚未得到完整再生植株; 牡丹分生结节培养以黄化嫩茎和叶柄薄层为外植体,前人已初步建立了紫斑牡丹和牡丹芍药组间杂种——伊藤的分生结节发生体系,并实现了不定芽和叶状体的分化,但分化率极低,且未能获得再生植株,还发现牡丹分生结节中含有丰富的芍药苷和丹皮酚,这证实了其在药用成分生产中的应用潜力。(2) 牡丹体细胞胚发生途径包括直接发生和间接发生,前人通过筛选基因型、培养基和发育时期等实现了多个牡丹品种的体细胞胚直接发生,但体细胞胚萌发率低,且再生成苗困难; 间接发生途径以叶柄为外植体,已诱导得到愈伤组织并获得体细胞胚,但体细胞胚发生率极低。(3) 牡丹无菌短枝扦插技术历经约 30 年的研究,完善的启动、增殖、生根和驯化移栽技术体系已经建立,但该技术仍无法实现产业化应用,主要原因在于试管苗的顶芽休眠、两步生根成本高以及移栽成活率低。2) 胚珠和胚离体培养成苗: 分别以完成器官分化的胚珠(花后 60 天) 和成熟胚(花后 90 天) 为外植体,前人通过筛选植物生长调节剂建立了紫斑牡丹和杨山牡丹的胚珠和胚离体培养体系,并获得了组培苗,但组培苗在驯化移栽阶段死亡。3) 花药(花粉) 离体培养成苗: 以处于第 1 次分裂期的花粉为外植体可诱导得到花粉胚,培养过程中不同发育状态的花粉胚表现出不同的器官分化能力,其中聚集成簇的花粉胚无法分化出芽和根,而独立生长的花粉胚可分化出根系,但无法分化出芽。综上,牡丹组织培养研究目前仍处于应用基础研究的初级阶段,后期研究一方面需要针对褐化、器官间接发生困难以及试管苗的生根和移栽问题进行技术优化,另一方面亟需从生理甚至是分子的层面对组织培养中的褐化、器官发生和顶芽休眠机理等展开研究,从而为组织培养技术的优化提供理论支撑。

关键词: 牡丹; 组织培养; 器官发生; 体细胞胚; 无菌短枝扦插; 胚珠培养; 胚培养; 花药(花粉) 培养
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Research Advances in Tissue Culture of Tree Peony

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Abstract: The tree peony(*Paeonia* Sect. *Moutan*), native to China, is famous for its ornamental value, medical use and edible oil production; an effective tissue culture system is an urgent requirement for its breeding and propagation. Tissue culture of tree peony has been extensively studied during the past half-century, and the main advances include the following 3 aspects: 1) Organ and tissue regeneration culture: there are 3 ways for regeneration including organogenesis, somatic embryogenesis and *in vitro* shoot proliferation. (1) Tree peony organogenesis includes callus culture and meristematic nodules culture. Vegetative and reproductive organs can both be used as explants to induce callus, but it is very difficult to induce shoots from callus, and a complete plant is still not obtained. With the yellowing stem segments and thin-layer petiole as explants, effective meristematic nodules culture systems have been developed for *P. rockii* and *P. itoh* by selecting plant growth regulators and improving culture medium. Adventitious buds and thallus were induced from the meristematic nodules; however, a complete plant is not obtained. In addition, the meristematic nodules of tree peony

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have been found to contain abundant peoniflorin and paeonol which indicate its value in pharmaceutical ingredients production. (2) Somatic embryogenesis: tree peony somatic embryogenesis includes direct and indirect ways. Somatic embryos have been induced in many tree peony cultivars by selecting genotype, culture medium and embryo development stage; however, the somatic embryo has been found difficult for germination and plant regeneration. Tree peony petioles have been used as explants to induce callus though indirect organogenesis, and the somatic embryo have been induced from callus with however extremely low induction rate. (3) *In vitro* shoot proliferation: *in vitro* shoot proliferation of tree peony has been studied for nearly 30 years. Effective initiation, multiplication, rooting and acclimatization system have been established; however, the protocol is unviable for commercial use due to the dormancy of the rooted shoots, the high cost of the two-step rooting protocol and the poor survival rate during acclimatization. 2) Ovule and embryo culture: Using mature ovule (60 days after flowering) and mature embryo (90 days after flowering) as explants, ovule and embryo culture systems have been developed for *P. rockii* and *P. ostii* by selecting plant growth regulators, and complete plants have been obtained which however died during acclimatization. 3) Anther (pollen) culture: Pollen at the first stage of division is the best explant for anther (pollen) culture. In tissue culture, the pollen embryos in different development states vary in organ differentiating capacity. The clustered pollen embryo can not produce shoots or roots, while the pollen embryo developing independently can produce roots, however without shoots produced. In conclusion, tissue culture of tree peony is still in the primary stage of applied basic research, the future technology research should focus on browning, the difficulties in indirect organogenesis, and the rooting and acclimatization problems to optimize the protocol. Meanwhile studies on the physiological and the molecular mechanism of the browning, organ differentiation, apical shoot dormancy, etc. is essential, in order to provide a theoretical basis for the establishment of tree peony tissue culture system.

Key words: tree peony; tissue culture; organogenesis; somatic embryo; *in vitro* shoot proliferation; ovule culture; embryo culture; anther (pollen) culture

牡丹(*Paeonia* Sect. *Moutan*)为芍药科(Paeoniaceae)芍药属(*Paeonia*)落叶亚灌木,是我国特有的名贵观赏兼药用植物,至今已有 1 600 余年的栽培历史(Cheng, 2007)。近年来,牡丹籽的油用价值逐渐被发掘,国务院于 2014 年颁发了《关于加快木本油料产业发展的意见》,首次把油用牡丹列为国家重点发展的木本油料作物,自此,牡丹成为了一种集观赏、药用和食用保健于一身的重要经济植物,拥有巨大市场需求。

目前,远缘杂交是牡丹育种的主要方式,然而杂交获得的种子极易败育,且 1 个牡丹新品种从授粉、采种、播种、开花、性状稳定、筛选、定名、申报到最终推广需要经历约 10 余年的时间,因此,杂交败育和育种周期长是牡丹育种的主要难题(Cheng, 2007)。分株和嫁接是商业化繁殖牡丹的主要途径,但分株法的繁殖系数低、周期长,每 3 ~ 4 年才能分株 1 次(李嘉珏等, 2011);嫁接法繁琐耗时,需要耗费大量砧木,且受时间限制,最佳嫁接时机为 8 月末—10 月初,此时嫁接成活率高达 80% ~ 90%,其余时间嫁接成活率极低(≤50%)(王世端, 1966)。综上,牡丹的传统繁殖方法繁殖效率低、周期长,这严重限制了牡丹的育种和繁殖工作的开展。

组织培养技术因其微型繁殖系数高、周期短、可保持母株优良性状等优势,近几十年来迅速发展,已被广泛应用于植物快速繁殖、植株脱毒、新品种选育、人工种子和药用成分生产中,并为植物基因工程创造了前提条件。牡丹组织培养技术研究始于 1965 年(Partanen, 1965),虽然李萍等(2007)、Qin 等(2012a)、Da Silva 等(2012)曾分别对牡丹组织培养研究进展进行过综述,但近年来,随着油用牡丹产业价值逐渐被发掘,牡丹组织培养迎来了一股研究热,在涉及技术领域的广度和深度方面都取得了诸多新进展。鉴于此,本文围绕器官组织培养再生、胚珠和胚离体培养成苗以及花药(花粉)离体培养成苗这 3 个方面,全面综述了自 1965 年至今约半个世纪的牡丹组织培养技术研究进展,并分析了该技术存在的主要问题及今后的研究方向,以期为牡丹组织培养技术的系统建立提供参考和借鉴,为加速其育种和繁殖进程奠定基础。

1 器官组织培养再生

植物组织培养再生途径主要有 3 种:器官发生、体细胞胚发生和无菌短枝扦插途径(罗士韦等, 1988),本文从这 3 个方面对牡丹的器官组织培养再生研究展开综述。

1.1　器官发生途径

1.1.1　愈伤组织培养　利用牡丹的各种繁殖器官(花瓣、花丝、花药、子叶等)和营养器官(叶片、叶柄、茎段等)为外植体皆可诱导得到愈伤组织(表 1)。Demoise 等(1969)以牡丹(*P. suffruticosa*)合子胚为外植体,首次诱导得到愈伤组织,但未能实现器官发生。张健欣(2015)以紫斑牡丹(*P. rockii*)为研究对象,发现牡丹繁殖器官愈伤组织诱导能力由高到低依次为胚轴 > 子叶 > 花瓣 > 雄蕊 > 心皮 > 柱头 > 花丝 > 花药,牡丹营养器官愈伤组织诱导能力由高到低依次为无菌苗叶柄 > 无菌苗叶片 > 大田叶柄 > 大田叶片。虽然大量牡丹品种已实现愈伤组织诱导,但由愈伤组织再生完整植株难度较大,例如 Beruto 等(2004)和王军娥等(2008)成功地由愈伤组织诱导获得不定芽,但诱导率较低,分别为 30.5% 和 50.0%,且未获得再生植株; 李玉龙等(1984)和朱向涛等(2015)由愈伤组织诱导获得不定芽,不定芽分化率为 20% ~40%,并且将不定芽诱导生根得到再生植株,但生根率仅为 10%。

1.1.2　分生结节培养　离体培养的植物组织有时会进入一种特殊而稳定的状态,从外部形态上,它是一种质地紧实的细胞团结构; 从内部结构上,它包括分生细胞、薄壁细胞和维管单元这 3 种细胞类型,并包含皮层和表皮 2 层组织(Mccown *et al.*, 1988)。分生结节是一种不同于体细胞胚,但与植物体细胞胚具有等同价值的又一种植物体外再生方式,并在植物微繁殖和次生代谢产物的生产方面具有重要价值(Mccown *et al.*, 1988)。牡丹分生结节培养研究起步较晚,且现有研究较少(表 1)。Gildow 等(2006)首次以牡丹黄化嫩茎为外植体,诱导获得分生结节,但仅分化出不定根。钟原(2011)以紫斑牡丹的叶柄薄层为外植体诱导获得愈伤组织,然后由愈伤组织诱导发生分生结节,初步建立了牡丹分生结节增殖培养体系,并测定了分生结节中的芍药苷和丹皮酚含量,证实了分生结节培养在牡丹药用成分生产中的应用潜力。随后,Qin 等(2012b)进一步优化了牡丹分生结节的发生体系,并对其分生结节的发生发育过程进行了组织切片观察和阶段划分,实现了 'Golden Era'(*P.* × *lemoinei* 'Golden Era')和伊藤杂种 'Barzella'(*P. itoh* 'Barzella')分生结节的诱导和增殖,且 'Golden Era' 的分生结节进一步分化不定芽,分化率为 16%,但未能获得再生植株。

1.2　体细胞胚发生途径

牡丹体细胞胚发生主要以胚为外植体,营养器官(叶柄、叶片和根等)也可用于诱导体细胞胚,但难度较大且尚未获得再生植株(表 1)。何桂梅(2006)以牡丹合子胚为外植体,研究发现基因型、诱导培养基和发育时期是影响体细胞胚发生的重要因素: 紫斑牡丹比杨山牡丹(*P. ostii*)更容易诱导体细胞胚; 成熟胚的诱导多表现为直接器官分化,幼胚和近成熟胚诱导体细胞胚效果较佳,可直接由胚轴分化体细胞胚,但只有紫斑牡丹的体细胞胚再生成苗,但成苗率较低,仅为 5.8%。随后,周秀梅(2008)进一步优化了紫斑牡丹体细胞胚的诱导培养基,其诱导率高达 63.3%,并获得了体细胞胚再生植株。朱向涛等(2012)以凤丹牡丹的胚、胚轴和子叶为外植体诱导体细胞胚,发现花后 100 天的胚诱导效果最佳,并进一步筛选出适宜其体细胞胚诱导的培养基,诱导率高达 38.3%。殷丽青等(2013)研究发现,在培养基中添加 4.0 mg · L^{-1} 毒莠定可显著提高牡丹胚性愈伤组织和体细胞胚的诱导率。

1.3　无菌短枝扦插途径

目前在牡丹组织培养技术中,以鳞芽为外植体的无菌短枝扦插技术自李玉龙等(1984)首次报道以来获得了最广泛深入的研究,其在外植体、启动和增殖培养、生根培养和驯化移栽这 4 个方面都取得了较大进展。

1.3.1　外植体　基因型、外植体类型和取材时期是决定启动培养的关键因素(表 1)。在相同的环境条件下,早花品种萌动早,芽的诱导表现良好,中花次之,晚花品种萌动最晚(刘磊等, 2008)。前人比较了不同类型外植体(顶芽、腋芽、萌蘖芽、萌生条)的启动培养效果,发现萌蘖芽的培养效果最好,萌发率最高(孔祥生等, 1998; 张桂花等, 2001; Beruto *et al.*, 2004)。早春 2 月为最佳取材时间,此时的芽刚经过低温休眠,芽体充分分化,营养物质累积充分,鳞片紧密,从而接种污染率低,成活率高,萌发快且生长健壮(孔祥生等, 1998; 张桂花等, 2001)。鳞芽作为无菌短枝扦插的主要外植体,鳞芽的表面灭菌是该技术的首要步骤。前人从灭菌剂的种类和灭菌时间这 2 方面对牡丹鳞芽表面灭菌技术进行了研究,发现 75% 酒精灭菌 25 ~30 s,再以 0.1% 升汞灭菌 8 ~ 10 min 效果最佳,此时外植体存活率高达 100%(邱金梅, 2010)。

1.3.2　启动和增殖培养　WPM 是牡丹增殖培养最常用的基本培养基,调节其中 Ca^{2+} 浓度、NO_3^--N/NH_4^+-N 以及植物生长调节剂浓度是促进试管苗增殖和生长的有效手段(表 1)。提高 WPM 培养基中的 Ca^{2+} 浓度不仅能有效防止继代芽的茎尖坏死,还能促进试管苗的增殖和生长(Bouza *et al.*, 1994a; Li

et al., 2008; 文书生等, 2016)。提高培养基中 NO_3^--N/NH_4^+-N 的比值可促进牡丹试管苗的增殖和生长(Li *et al.*, 2008; 文书生等, 2016)。Bouza 等(1994a)比较了 5 种传统细胞分裂素[玉米素(Zt)、异戊烯基腺嘌呤(2iP)、激动素(KT)、异戊烯腺苷(iPA)和 BA]对丛生芽诱导的影响,发现 BA 效果最佳。BA 通常与 GA_3 配合使用以进一步提高增殖率,并促进茎段伸长(李玉龙等, 1984; 孔祥生等, 1998; Wen *et al.*, 2016a; 王新等, 2016),但同时需提高培养基中 Ca^{2+} 浓度以防止 GA_3 引发茎尖坏死(Bouza *et al.*, 1994a; Li *et al.*, 2008)。最新研究发现,在'正午'牡丹(*P.* ×*leimonei* 'High Noon')无菌短枝扦插的增殖阶段以新型细胞分裂素 *meta*-Topolin(*m*T)替代 BA,*m*T-GA_3 与 BA-GA_3 组合对试管苗的增殖和生长具有基本等同的促进作用(Wen *et al.*, 2016b)。综上,WPM [$Ca(NO_3)_2$ 1 544 ~ 1 668 mg·L^{-1}] + BA/*m*T + GA_3 是牡丹增殖培养阶段最常用的培养基,但培养过程中需根据品种筛选最佳 BA/*m*T 和 GA_3 浓度配比。

1.3.3 生根培养 牡丹试管苗生根的影响因素较为复杂,而培养基和生根方式是决定生根效果的关键(表 1)。牡丹试管苗生根最常用的基本培养基为 1/2MS(Bouza *et al.*, 1994b; 1994c; 孔祥生等, 1998; Wen *et al.*, 2016a; 2016b; 王新等, 2016)。牡丹试管苗茎内不存在潜伏根原基(贺丹等, 2011; 贾文庆等, 2013),IBA 是诱导其不定根发生的最有效生长素,采用速蘸生根法,试管苗生根率较高,且根系良好(李玉龙等, 1984)。随后,Bouza 等(1994c)比较了 3 种生根方式(一步生根法、速蘸生根法、两步生根法)的生根效果,发现两步生根法效果最佳,即先将试管苗于含高浓度 IBA(15 mg·L^{-1})的培养基上暗培养 10 天以诱导根原基,然后转至含活性炭、无植物生长调节剂的培养基培养以促进根的伸长。Beruto 等(2007)进一步改良了两步生根法,即在生根前先将无根苗进行冷处理(2 ℃,7 天)以促进生根。目前,改良的两步生根法是牡丹试管苗生根的最常用方法,但不同品种根诱导适宜的 IBA 浓度和诱导时间差异较大,例如牡丹'Mme de Vatry'(*P. suffruticosa* 'Mme de Vatry')为 15 mg·L^{-1} IBA 暗培养 10 天(Bouza *et al.*, 1994b),'正午'为 1 mg·L^{-1} IBA 暗培养 30 天(文书生等, 2016),'凤丹白'(*P. ostii* 'Fengdan Bai')为 2 mg·L^{-1} IBA 暗培养 30 天(王新等, 2016)。在上述改良的两步生根法基础上,Wen 等(2016a)发现于含 IBA 的根诱导培养基中再添加腐胺(PUT)可有效促进牡丹试管苗生根,5.0、1.0 和 1.0 mg·L^{-1} PUT 可分别将'乌龙捧盛'(*P. suffruticosa* 'Wulong Pengsheng')、'锦袍红'(*P. suffruticosa* 'Jinpao Hong')和'正午'的生根率由 30% ~ 50% 提高到 80% ~ 100%。综上,诱导牡丹试管苗生根的常用方法为:先以 1/2MS + IBA + PUT 诱导不定根发生,再转入 1/2MS + 活性炭以促进不定根的伸长,但需根据品种来筛选最佳的 IBA 浓度、PUT 浓度和根诱导时间。

此外,最新研究还发现在牡丹无菌短枝扦插的增殖阶段以 *m*T 替代 BA,*m*T 处理的试管苗生根率和移栽成活率更高,这表明在增殖阶段以新型细胞分裂素 *m*T 替代 BA 是提高生根率和移栽成活率的有效途径(Wen *et al.*, 2016b),但 *m*T 的有效性仍有待在更多牡丹品种中验证,且 *m*T 在牡丹无菌短枝扦插中的作用机理也值得深入研究。

1.3.4 驯化移栽 Bouza 等(1992)首次提出在牡丹无菌短枝扦插的根诱导阶段产生了顶芽休眠,未解除休眠的试管苗移栽后不生长,并逐渐死亡。前人尝试以冷处理解除牡丹试管苗的顶芽休眠,却发现冷处理无法彻底解除休眠,移栽 60 天后试管苗再次进入休眠状态,生长停滞,并逐渐死亡(Bouza *et al.*, 1994d),近期,在牡丹'乌龙捧盛'、'锦袍红'、'正午'和'凤丹白'试管苗的顶芽休眠研究中也得出了类似结论(Wen *et al.*, 2016a; 2016b; 王新等, 2016; 文书生等, 2016)。牡丹试管苗移栽最佳基质为珍珠岩、蛭石和草炭土等比混合(王新等, 2016),且于移栽过程中接种适宜的丛枝菌根真菌可大幅提高移栽成活率,并促进其后期生长(Wen *et al.*, 2016a)。

2 胚珠和胚离体培养成苗

胚珠和胚培养可使牡丹种胚快速萌发成苗,提高其种子萌发率,缩短萌发时间,从而加速育种进程。何桂梅等(2006)首次对紫斑牡丹和杨山牡丹胚珠培养进行了报道,发现发育早期的胚珠(花后 48 天以内)极难培养成功,而完成器官分化的胚珠(花后 60 天)可培养成苗,但成苗率极低(≤ 21.4%)。胚培养是指将种胚接种在无菌条件下培养使之直接发育成幼苗的技术,成熟胚(花后 90 天)的培养效果优于幼胚(花后 65 天内),紫斑牡丹和杨山牡丹成熟胚的成苗率分别为 71.6% 和 63.6%(何桂梅等, 2006)。如何协调上、下胚轴的生长是牡丹胚培养的关键,其中植物生长调节剂发挥重要作用:BA 可促进上胚轴伸长,但对下胚轴伸

表 1　牡丹器官组织培养再生研究

Tab. 1　Tree peony regeneration through organ and tissue culture

种及品种 Species or cultivar	外植体 Explant	培养基和植物生长调节剂 Medium and plant growth regulators/(mg · L^{-1})	培养结果 Result	参考文献 Reference
1. 器官发生途径：Ⅰ. 愈伤组织诱导；Ⅱ. 愈伤组织增殖；Ⅲ. 不定芽分化；Ⅳ. 不定芽生根 1. Organogenesis: Ⅰ. Callus induction; Ⅱ. Callus proliferation; Ⅲ. Adventitious shoot induction; Ⅳ. *in vitro* rooting				
牡丹 *Paeonia. suffruticosa*	胚 Embryo	基本培养基 Basal medium(Steeves *et al.*,1955) +2,4-D 0.2	愈伤组织 Callus	Demoise *et al.*,1969
牡丹‘青龙卧墨池’ *P. suffruticosa*‘Qinglong Womochi’	叶片、叶柄 Leaves, petiole	Ⅰ, Ⅱ. MS + BA 2 + NAA 0.1 ~ 0.5 Ⅲ. MS + BA 2 + IAA 0.2 ~ 0.5	不定芽丛 Adventitious shoot clusters	李玉龙等, 1984 (Li *et al.*,1984)
凤丹牡丹‘凤丹白’,牡丹‘白珍珠’ *P. ostii*‘Fengdan Bai’; *P. suffruticosa*‘White Pearl’	花丝、花瓣 Filament, petal	Ⅰ. 花丝 Filament: MS + 2iP 24.60; 花瓣 Petal: MS + TDZ 2.27 + 2,4-D 9.05 Ⅱ. MS + TDZ 0.5	叶状体 Thallus	Beruto *et al.*,2004
凤丹牡丹 *P. ostii*	子叶、胚轴 Cotyledon, hypocotyl	I. 1/2 Improved WPM + 2,4-D 1 + 6-BA 1.0	愈伤组织 Callus	郎玉涛等, 2007 (Lang *et al.*,2007)
法国牡丹‘金阁’ *P.* × *lemoinei*‘Souvenir de Maxime Comu’	叶柄 Petiole	I, Ⅱ. 2,4-D 2.0 + 6-BA 1.0 + NAA 1 + TDZ 0.5 III. WPM + 2,4-D 0.25 + TDZ 0.5 IV. 1/2MS + 6-BA 2 + NAA 0.5	生根苗 Rooted shoots	王军娥等, 2008 (Wang *et al.*,2008)
凤丹牡丹 *P. ostii*	花药 Anther	I. MS + 2,4-D 2 + 6-BA 1.5 + NAA 1	愈伤组织 Callus	朱向涛等, 2010 (Zhu *et al.*,2010)
凤丹牡丹 *P. ostii*	茎段 Stem segment	I, Ⅱ. 1/2MS + 2,4-D 0.2 + 6-BA 2 + NAA 0.3 III. MS + KT 0.2 + BA 0.3 + NAA 0.1 IV. 1 /2MS + IBA 0.2	完整植株 Plants	朱向涛等, 2015 (Zhu *et al.*,2015)
牡丹 *P. suffruticosa*	茎段 Stem segment	I. SH-M + 2,4-D 0.1 分生结节的诱导 Meristematic nodules induction: SH/1/2SH/2SH/SH-M + 2,4-D 0.2 + KT 0.1 分生结节分化 Meristematic nodules differentiation: SH/1/2SH/2SH/SH-M	分生结节分化不定根 Meristematic nodules differentiated adventitious roots	Gildow *et al.*,2006
紫斑牡丹‘高原圣火’、‘桃花镶玉’ *P. rockii*‘Gaoyuan Shenghuo’,‘Taohua Xiangyu’	叶片、叶柄 Leaves, petiole	I. SH + 2,4-D 0.1 ~ 0.5 分生结节的诱导 Meristematic nodules induction: SH + 2,4-D 0.1 ~ 2.0, 再转 Transferred to SH + 2,4-D 0.5 + BA 4	分生结节 Meristematic nodules	钟原, 2011 (Zhong, 2011)
牡丹‘洛阳红’、‘乌龙捧盛’,凤丹牡丹‘凤丹白’,紫斑牡丹‘红莲’,美国牡丹‘正午’、‘Golden Era’,伊藤杂种‘Bartzella’ *P. suffruticosa*‘Luoyang Hong’,‘Wulong Pengsheng’; *P. ostii*‘Fengdan Bai’; *P. rockii*‘Honglian’; *P.* × *lemoinei*‘High Noon’,‘Golden Era’; *P. itoh*‘Bartzella’	叶柄 Petiole	I. SH + 2,4-D 1 分生结节的诱导 Meristematic nodules induction: SH + 2,4-D 0.2 ~ 0.5 + BA 2 ~ 6 分生结节分化 Meristematic nodules differentiation: SH + TDZ 0.5 + NAA 0.25	‘Bartzella’的分生结节分化出不定芽,其余品种仅诱导获得分生结节 Meristematic nodules of ‘Bartzella’ differentiated adventitious shoots, while the other cultivars didn’t	Qin *et al.*,2012b

续表 Continued

种及品种 Species or cultivar	外植体 Explant	培养基和植物生长调节剂 Medium and plant growth regulators/(mg·L^{-1})	培养结果 Result	参考文献 Reference
2. 体细胞胚发生途径：Ⅰ. 体细胞胚诱导；Ⅱ. 体细胞胚增殖；Ⅲ. 体细胞胚萌发成苗 2. Somatic embryogenesis: Ⅰ. Somatic embryo induction; Ⅱ. Somatic embryo proliferation; Ⅲ. Somatic embryo germination				
紫斑牡丹'书生捧墨'，凤丹牡丹'凤丹白'，日本牡丹'岛锦'、'连鹤' *P. rockii* 'Shusheng Pengmo'; *P. ostii* 'Fengdan Bai'; *P.* ×*suffruticosa* 'Daojin', 'Lianhe'	胚珠、胚 Ovule, embryo	Ⅰ. 1/2MS + BA 0.5 Ⅱ. 1/2MS + IBA 0.1 ~ 0.4 Ⅲ. 1/2MS	完整植株 Plants	何桂梅，2006 (He *et al.*,2006)
紫斑牡丹'书生捧墨' *P. rockii* 'Shusheng Pengmo'	胚 Embryo	Ⅰ. Improved MS + BA 0.5 Ⅱ. Improved MS + BA 0.5 Ⅲ. Improved MS + 活性炭 Activated carbon 100	完整植株 Plants	周秀梅，2008 (Zhou *et al.*,2008)
凤丹牡丹 *P. ostii*	胚、胚轴、子叶 Embryo, hypocotyl, cotyledons	Ⅰ. MS + 2,4-D 2 + BA 2	体细胞胚 Somatic embryos	朱向涛等，2012 (Zhu *et al.*,2012)
凤丹牡丹 *P. ostii*	胚、叶柄、根 Embryo, petiole, root	愈伤组织的诱导和增殖 Callus induction and proliferation: Improved MS + BA 0.5 Ⅰ,Ⅱ. Improved MS + BA 0.5 Ⅲ. Improved MS + BA 0.5 + NAA 0.1	胚培养获得完整植株，叶柄和根培养获得愈伤组织 Plants were obtained from embryo culture, but only callus were obtained from petiole and root culture	殷丽青等，2013 (Yin *et al.*,2013)
紫斑牡丹'美髯公'、'粉荷'、'粉冠彩' *P. rockii* 'Mei Rangong', 'Fenhe', 'Fenguan Cai'	叶柄、叶片 Petiole, leaves	愈伤组织的诱导 Callus induction: MS + BA 2 + NAA 0.4 愈伤组织的增殖 Callus proliferation: MS + 2,4-D 0.5 + NAA 1.5 Ⅰ. MS + BA 4 + 2,4-D 0.4	体细胞胚 Somatic embryos	张健欣，2015 (Zhang,2015)
3. 无菌短枝扦插途径：Ⅰ. 启动培养；Ⅱ. 增殖培养；Ⅲ. 生根培养；Ⅳ. 驯化移栽 3. *In vitro* shoot proliferation: Ⅰ. Initiation; Ⅱ. Multiplication; Ⅲ. *in vitro* rooting; Ⅳ. Acclimatization and transplanting				
牡丹'青龙卧墨池'，日本牡丹'18号' *P. suffruticosa* 'Qinglong Womochi'; *P.* ×*suffruticosa* '18th'	腋芽 Axillary bud	Ⅰ,Ⅱ. 1/2MS + KT 0.2 ~ 1 + BA 0.5 ~ 1 + GA_3 0.1 ~ 0.5 Ⅲ. IBA 50 ~ 100 处理 2 ~ 3 h，再转入 1/2MS The shoots were treated with IBA 50 ~ 100 for 2 ~ 3 h, then transferred to 1/2MS Ⅳ. 移栽基质采用中性土壤 The rooted shoots were transplanted to neutral soil	完整植株 Plants	李玉龙等，1984 (Li *et al.*,1984)
牡丹 *P. suffruticosa*	茎段 Stem segment	Ⅰ,Ⅱ. Lepoivre + BA 1 + GA_3 0.1 Ⅲ. Lepoivre 4 ℃暗培养 4 周，再转入 Lepoivre + IBA 0.1 The shoots were cultured on Lepoivre for 4 weeks in the dark, then transferred to Lepoivre + IBA 0.1 Ⅳ. 移栽基质为草炭和珍珠岩等体积混合 The rooted shoots were transplanted to peat/perlite(1/1, *V/V*)	完整植株 Plants	Kunneman *et al.*,1989; Albers *et al.*,1991
紫斑牡丹 *P. rockii*	茎段 Stem segment	Ⅰ,Ⅱ. MS + BA 1 + 2ip 1 Ⅲ. MS + IBA 1	生根苗 Rooted shoots	Harris *et al.*,1991

续表 Continued

种及品种 Species or cultivar	外植体 Explant	培养基和植物生长调节剂 Medium and plant growth regulators/(mg · L^{-1})	培养结果 Result	参考文献 Reference
牡丹'Madame de Vatry' *P. suffruticosa*'Madame de Vatry'	腋芽 Axillary bud	I,II. MS + BA 1 III. 根诱导 Root induction:MS + IBA 15 根形成 Root development:MS + 活性炭 Activated carbon 3 000	生根苗 Rooted shoots	Bouza *et al*.,1992
牡丹'Madame de Vatry' *P. suffruticosa*'Madame de Vatry'	腋芽 Axillary bud	I,II. MS($CaCl_2$880) + BA 1	丛生芽 Shoot clusters	Bouza *et al*.,1993
牡丹'Madame de Vatry' *P. suffruticosa*'Madame de Vatry'	腋芽 Axillary bud	I,II. MS($CaCl_2$880) + BA 1 + $GA_3$0.5	丛生芽 Shoot clusters	Bouza *et al*.,1994a
牡丹'Madame de Vatry' *P. suffruticosa*'Madame de Vatry'	腋芽 Axillary bud	I,II. MS($CaCl_2$880) + BA 1 + $GA_3$0.5 ~ 1 III. 根诱导 Root induction:1/2MS + IBA 15 根形成 Root development:MS + 活性炭 Activated carbon 3 000	提出两步生根法,并获得生根苗 Two-step rooting protocol was established, and rooted shoots were obtained	Bouza *et al*., 1994b; 1994c; 1994d
牡丹 *P. suffruticosa*	组培苗 *In vitro* shoot	II. MS + BA 1 + NAA 1	丛生芽 Shoot clusters	储成才等, 1993 (Chu *et al*.,1993)
牡丹'姚黄'、'胭脂红'、'夜光白'、'洛阳红' *P. suffruticosa* 'Yaohuang', 'Yanzhi Hong', 'Yeguang Bai', 'Luoyang Hong'	休眠芽 Dormant bud	I,II. 改良 MS + BA 1 + $GA_3$0.5 III. 1/2MS + IBA 1	完整植株 Plants	孔祥生等, 1998 (Kong *et al*.,1998)
牡丹'Comtesse de Tuder' *P. suffruticosa*'Comtesse de Tuder'	茎段 Stem	I,II. MS + BA 1 + NAA 0.2	丛生芽 Shoot clusters	Černá *et al*.,2001
牡丹'黑花魁'、'豆绿'、'金星雪浪'、'大胡红' *P. suffruticosa*'Hei Huakui', 'Dou Lv', 'Jinxing Xuelang', 'Da Huhong'	腋芽、顶芽 Axillary bud, terminal bud	I,II. MS + BA 0.5 ~ 1 + NAA 0.1 ~ 0.2	丛生芽 Shoot clusters	张桂花等, 2001 (Zhang *et al*.,2001)
紫斑牡丹 *P. rockii*	休眠芽 Dormant bud	I,II. MS + BA 1 + NAA 0.5 + 2,4-D 0.5	丛生芽 Shoot clusters	陈怡平等, 2003 (Chen *et al*.,2003)
牡丹'白玉'、'石元白'、'白珍珠'、'姚黄'、'Huang Hu'、'Orange Yellow'、'金殿'、'胡红'、'大金凤'、'Fish Scale Pink'、'状元红'、'紫二乔'、'First Red'、'Red Diamond'、'珠沙垒'、'Old Pink',日本牡丹'Orange'、'Red',紫斑牡丹'雪莲',凤丹牡丹'凤丹白' *P. suffruticosa* 'Baiyu', 'Shiyuan Bai', 'White Pearl', 'Yaohuang', 'Huang Hu', 'Orange Yellow', 'Golden Palace', 'Huhong', 'Da Jinfeng', 'Fish Scale Pink', 'Zhuangyuan Hong', 'Zi Erqiao', 'First Red', 'Red Diamond', 'Zhusha Lei', 'Old Pink'; *P.* ×*suffruticosa* 'Orange', 'Red'; *P. rockii* 'Xue Lian'; *P. ostii*'Fengdan Bai'	腋芽 Axillary bud	I,II. Improved WPM + 柠檬酸 Citric acid 75 + Ve 50 + BA 1 III. 冷处理:改良 WPM,2 ℃暗处理 7 d Cold treatment:The shoots were cultured on improved WPM at 2 ℃ for 7 days in the dark 根诱导 Root induction:Improved WPM + 柠檬酸 Citric acid 75 + Ve 50 + IBA 1 根形成 Root development:WPM + 活性炭 Activated carbon 3 000 IV. 两步驯化法 Two-step acclimatization	开花植株 Flowering plants	Beruto *et al*.,2007;2004

续表 Continued

种及品种 Species or cultivar	外植体 Explant	培养基和植物生长调节剂 Medium and plant growth regulators/(mg·L^{-1})	培养结果 Result	参考文献 Reference
牡丹'青龙卧墨池' *P. suffruticosa* 'Qinglong Womochi'	腋芽 Axillary bud	I,II. MS + BA 0.5 ~ 1 III. MS + IBA 2	生根苗 Rooted shoots	张俊琦等, 2006 (Zhang *et al*.,2006)
牡丹'乌龙捧圣'、'迎日红' *P. suffruticosa* 'Wulong Pengsheng', 'Yingri Hong'	腋芽 Axillary bud	I. Improved MS + BA 1 + GA_3 0.2 ~ 0.5 I,II. Improved MS + BA 1 + GA_3 0.2 ~ 0.5	丛生芽 Shoot clusters	Li *et al*.,2008
牡丹'乌龙捧盛'、'肉芙蓉'、'鲁荷红'、'洛阳红'、'赵粉'、'胡红',凤丹牡丹'凤丹白' *P. suffruticosa* 'Wulong Pengsheng', 'Rou Furong', 'Lu Hehong', 'Luoyang Hong', 'Zhaofen', 'Huhong'; *P. ostii* 'Fengdan Bai'	腋芽、顶芽 Axillary bud, terminal bud	I,II. MS(WPM Ca^{2+}) + BA 2 + IAA 0.3	生根苗 Rooted shoots	何松林等, 2009 (He *et al*.,2009)
牡丹'乌龙捧盛' *P. suffruticosa* 'Wulong Pengsheng'	腋芽 Axillary bud	I,II. MS + BA 0.3 + NAA 0.3	丛生芽 Shoot clusters	Ding *et al*.,2010
牡丹'乌龙捧盛' *P. suffruticosa* 'Wulong Pengsheng'	腋芽 Axillary bud	I,II. WPM + BA 3 + IAA 0.2 III. Improved 1/2MS + IBA 4 + NAA 1	生根苗 Rooted shoots	刘会超等, 2010a (Liu *et al*.,2010a)
牡丹'乌龙捧盛' *P. suffruticosa* 'Wulong Pengsheng'	腋芽 Axillary bud	I,II. MS + BA 2 + NAA 0.5 + PVP 1 000 III. WPM + IBA 4	生根苗 Rooted shoots	Wang *et al*.,2012
牡丹'乌龙捧盛' *P. suffruticosa* 'Wulong Pengsheng'	组培苗 *In vitro* shoot	III. 根诱导 Root induction:1/2MS + IBA 3 + NAA 0.6 根形成 Root development:1/2MS	生根苗 Rooted shoots	贾文庆等, 2013 (Jia *et al*.,2013)
牡丹'乌龙捧盛'、'锦袍红';美国牡丹'正午' *P. suffruticosa* 'Wulong Pengsheng', 'Jinpao Hong'; *P.* ×*lemoinei* 'High Noon'	腋芽 Axillary bud	I. WPM($CaCl_2$ 290) + BA 1 II. WPM($CaCl_2$ 290) + BA 1 + GA_3 0.5 III. 根诱导 Root induction:1/2MS + IBA1 + PUT(腐胺,putrescine)1 ~ 5 根形成 Root development:1/2MS + 活性炭 Activated carbon 4 000 IV. 试管苗以 4 ℃暗处理 30 d 解除休眠,再移栽到草炭、珍珠岩和蛭石等体积混合基质,并接种丛枝菌根摩西球囊霉 1 g·株$^{-1}$ The shoots were cultured at 4 ℃ in dark for 30 days to release the dormancy, then rooted shoots were transplanted to vermiculite/peat/perlite(1/1/1, *V/V/V*) and inoculated with *Glomus mosseae* 1 g for each plant	完整植株 Plants	Wen *et al*.,2016a
美国牡丹'正午' *P.* ×*lemoinei* 'High Noon'	腋芽 Axillary bud	I. WPM($CaCl_2$ 290) + BA 0.5 + GA_3 0.2 II. WPM [$Ca(NO_3)_2$•$4H_2O$ 1 668] + BA 0.5 + GA_3 0.2 III. 复壮培养 Rejuvenation culture:1/2MS($CaCl_2$ 296) + 活性炭 Activated carbon 0.5, 20 d 根诱导 Root induction:1/2MS($CaCl_2$ 296) + IBA 1 + PUT 1 根形成 Root development:1/2MS($CaCl_2$ 296) + 活性炭 Activated carbon 4 000	开花植株 Flowering plants	文书生等, 2016 (Wen *et al*.,2016)

续表 Continued

种及品种 Species or cultivar	外植体 Explant	培养基和植物生长调节剂 Medium and plant growth regulators/(mg·L^{-1})	培养结果 Result	参考文献 Reference
		IV. 试管苗以 4 ℃暗处理 30 d 解除休眠，再移栽到草炭、珍珠岩和蛭石等体积混合基质 The shoots were cultured at 4 ℃ in the dark for 30 days to release the dormancy, then the rooted shoots were transplanted to vermiculite/peat/perlite (1/1/1, *V*/*V*/*V*)		
美国牡丹'正午' *P.* × *lemoinei* 'High Noon'	腋芽 Axillary bud	I. WPM [$Ca(NO_3)_2 \cdot 4H_2O$ 1 112] + BA 1 II. WPM [$Ca(NO_3)_2 \cdot 4H_2O$ 1 112] + *m*T(*meta*-Topolin) 1 + GA_3 0.5 III. 冷处理 Cold treatment：4 ℃暗处理 8 d Cold treatment: The shoots were cultured at 4 ℃ in the dark for 8 days 根诱导 Root induction：1 /2MS($CaCl_2$296) + IBA 2 + PUT 1 根形成 Root development：1/2MS($CaCl_2$296) + 活性炭 Activated carbon 4 000 IV. 移栽基质为草炭、珍珠岩和蛭石等体积混合 The rooted shoots were transplanted to vermiculite/peat/perlite (1/1/1, *V*/*V*/*V*)	完整植株 Plants	Wen *et al*.,2016b
凤丹牡丹 *P. ostii*	腋芽 Axillary bud	I. WPM + BA 1 II. WPM [$Ca(NO_3)_2 \cdot 4H_2O$ 1 668] + BA 0.5 + GA_3 0.2 III. 根诱导 Root induction：1/2MS + IBA1 + PUT 1 根形成 Root development：1/2MS + 活性炭 Activated carbon 4 000 IV. 移栽基质为草炭、珍珠岩和蛭石等体积混合 The rooted shoots were transplanted to vermiculite/peat/perlite (1/1/1, *V*/*V*/*V*)	完整植株 Plants	王新等，2016 (Wang *et al*.,2016)

长有抑制作用；赤霉素(GA_3)可打破上胚轴休眠，促进上胚轴伸长，并促进胚根萌动，但导致弱苗现象(徐莉等，2017)；吲哚乙酸(IAA)促使种胚膨大，但仅上胚轴生长，不利于直接成苗(何桂梅，2006)；IBA 可诱导促进牡丹不定根发生(Bouza *et al.*，1994c)。为了提高牡丹胚培养效率，刘会超等(2010b)在牡丹胚培养过程中，首先利用 BA 诱导丛生芽提高繁殖系数，再将丛生芽切分成单芽利用 IBA 诱导生根，这种两步培养方式可分别促进牡丹上下胚轴伸长，但操作复杂、生根率较低。最新研究发现，单独利用 GA_3 打破牡丹上胚轴休眠，同时在培养基中添加活性炭吸附抑制生长的有害物质，并将 MS 的大量元素加倍是实现牡丹胚培养的有效途径，该方法可有效促进上胚轴伸长，并利用活性炭的吸附作用和培养基大量元素加倍避免发生弱苗现象(徐莉等，2017)。

3 花药(花粉)离体培养成苗

花药培养再生单倍体植株，然后经加倍获得纯合二倍体是花卉、林木染色体倍性创新的重要途径。早期关于牡丹花药(花粉)培养的研究主要由 Sunderland 工作组完成，他们发现处于第 1 次分裂期的花粉是最好的外植体，此时的花芽处于闭合状态，易于灭菌、消毒，且褐化程度最轻(Roberts *et al.*，1977)。组织培养过程中，花粉胚发育存在 2 种形式：1)花粉胚聚集成簇，即使在继代时将其分离培养，生长一段时间后还会聚集成团，偶尔会出现子叶体积大，但都没有形成正常的叶或枝。2)花粉胚独立生长，分化形成根和茎的两端，但进一步培养只形成根系，上部生长停滞，并最终死亡，对其幼苗采用根尖切片法检测染色体发现其为单倍体(Sunderland *et al.*，1975)。植物生长调节剂不是决定花粉胚诱导的关键因素，Sunderland 等(1975)以不含植物生长调节剂的 MS 液体培养基培养，成功获得了四川牡丹(*P. decomposita*)的花粉胚。后期，关于牡丹花药(花粉)培养的研究未能取得新的突破性进展，Zenkteler 等(1975)获得了牡丹多核花粉胚，其余研究仅获得了愈伤组织(Shoyama *et al.*，1990；陈怡平等，2001；朱向涛等，2010)。

4 结论与展望

牡丹组织培养技术历经约半个世纪的研究，在器官组织培养再生、胚珠和胚离体培养成苗以及花药(花粉)离体培养成苗这 3 个方面都取得了较大进展，但器官发生和花药(花粉)培养途径尚未获得完整植株，无菌短枝扦插途径以及胚珠和胚离体培养虽已获得完整植株，但离产业化以及育种的实践应用还有很大差距(图 1)，后期需围绕以下几个问题展开重点研究。

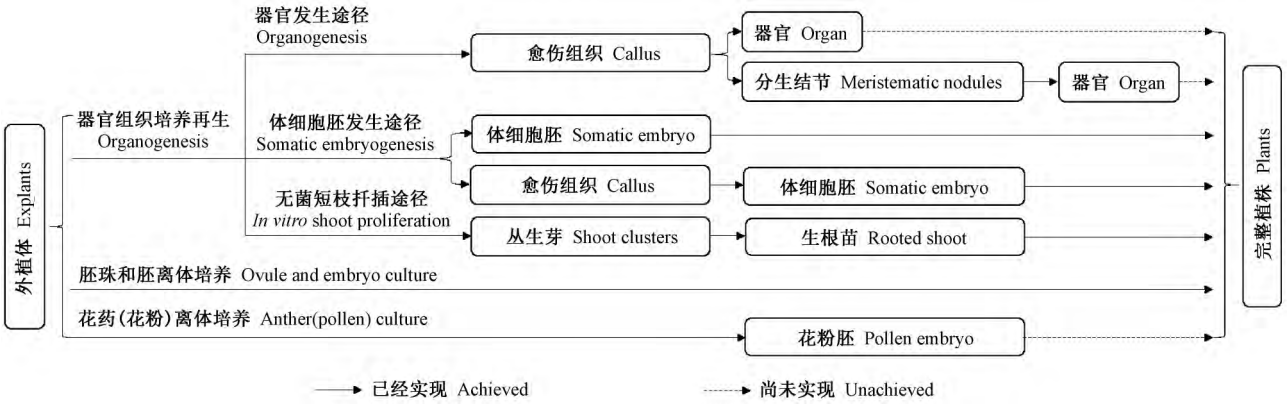

图 1 牡丹组织培养研究进展

Fig. 1 Advances of tree peony tissue culture

1)褐化：牡丹组培褐化严重，这对外植体的脱分化和再分化具有严重抑制作用。前人通过外植体预处理、调整培养基、控制培养条件、添加防褐剂等措施只能在一定程度上减轻褐化，建议后期研究在优化牡丹防褐技术的同时，进一步探究褐化发生机理从而为防褐技术的优化提供理论支撑。

2)器官间接发生困难：在牡丹的器官发生途径研究中，前人围绕培养基、植物生长调节剂、培养条件等方面展开了大量研究，然而愈伤组织和分生结节再生成芽的比率仍然极低，后期研究宜重点关注抑制牡丹器官分化的内在因素及其作用机理，例如内源激素水平、内源抑制物、DNA 甲基化水平等，深入探究上述问题对揭示牡丹器官发生的调控机制、构建促进牡丹器官发生的新方法皆具有重要意义。

3) 生根和移栽问题: 目前牡丹无菌短枝扦插技术已初步形成体系,后期研究建议着力于采取措施打破试管苗的顶芽休眠,简化生根流程,缩减生根和移栽成本,以推进牡丹无菌短枝扦插技术实现产业化应用。尤其,顶芽休眠作为牡丹试管苗移栽成活率低的主导因素,其诱导因素且如何作用,冷处理为何无法打破试管苗的顶芽休眠,都是值得深入研究的科学问题。

总之,牡丹组织培养研究目前仍处于应用基础研究的初级阶段,尽管由于一系列问题限制了其在牡丹的繁殖和育种实践中的应用,但其离体快繁的理论可行性已被证实,且已有部分研究获得了移栽成活的组培苗。随着相关基础理论研究的深入,牡丹组织培养的关键问题会不断得到解决,高效且稳定的植株再生及离体快繁技术体系将会建立,继而大幅提高牡丹的繁殖和育种工作效率。

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