

# 柑橘倍性操作技术与育种现状、难点及展望

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**摘 要:** 柑橘染色体倍性操作技术研究与应用取得了较大进展: 1. 三倍体、四倍体报道较多, 已有三倍体、四倍体新品种发布; 2. 部分四倍体作为砧木在控制树形、抗病、抗逆等方面有较好的表现; 3. 单倍体、双单倍体用于柑橘基因组研究获得重大进展。但柑橘倍性操作仍面临一些难点: 1. 童期长和珠心胚干扰影响多倍体新品种培育进度; 2. 单倍体和双单倍体获得依然较难; 3. 部分优质材料的育性低, 以其多倍体为亲本的杂交育种受到限制。针对童期较长的问题, 有必要应用缩短童期技术, 或利用短童期资源对童期长的材料进行改良。针对珠心胚干扰的影响, 应开发更多的单胚性四倍体材料, 还可结合胚抢救、分子标记鉴定等方法提高多胚性四倍体为亲本创制三倍体的效率。关于将来柑橘倍性操作与育种研究的建议: 1. 提高  $2n$  配子的产生频率, 以开发更多的多倍体类型, 尤其是单胚性品种多倍体创制; 2. 发挥多倍体作为砧木的优势, 促进砧木育种发展; 3. 继续诱导和利用柑橘单倍体、双单倍体, 以利于柑橘基因组、功能基因研究和育种; 4. 以异源多倍体创制技术为基础, 结合染色体操作技术培育仅带少量近缘物种基因组成分且具有目标性状的新种质。

**关键词:** 柑橘; 倍性操作; 四倍体; 三倍体; 单倍体

**中图分类号:** S 666

**文献标志码:** A

**文章编号:** 0513-353X (2021) 04-0791-20

## Ploidy Manipulation and Citrus Breeding: Current Status, Problems and Prospects

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**Abstract:** There have been some advanced progresses in ploidy manipulation of citrus. Firstly, numerous triploid and tetraploid have been reported, and some triploid and tetraploid varieties have been released. Secondly, Some tetraploid perform well in controlling tree shape, and being resistant to diseases and stresses when used as rootstocks. Thirdly, haploid and dihaploid were used in genome researches of *Citrus*, and great achievements had been harvested. Nevertheless, there are still some difficulties in ploidy manipulation of citrus. Firstly, long juvenile phase and nucellar embryo affect the efficiency of new

**收稿日期:** 2020-08-29; **修回日期:** 2021-02-04

**基金项目:** 重庆博士后基金特别资助项目 (XmT2018056); 重庆市基础与前沿研究计划项目 (cstc2016jcyjA0046); 重庆市科委民生科技创新专项 (cstc2016shmszx12080003)

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polyploid varieties breeding. Secondly, it is still difficult to obtain haploid and dihaploid, which limits the promotion of citrus genome researches and breeding. Thirdly, some polyploids with high qualities are lowly fertile, blocking the efficiency of breeding by using these materials. Regarding the problem of long juvenile phase in citrus polyploidy breeding, it is necessary to use new technologies of shortening it or to take advantage of germplasm resources with short juvenile phase. For overcoming the interference of nucellar embryo, it is necessary to develop more monoembryonic tetraploids. When using polyembryonic tetraploids as parents to produce triploids, embryo rescue, and molecular marker can be used to improve the breeding efficiency. At last, the authors suggested that ploidy manipulation in citrus could be extending in the following aspects: firstly, improving the frequency of  $2n$  gamete production in order to develop more polyploid, especially monoembryonic types; secondly, the advantages of polyploid being used as rootstock and polyploid rootstock should be brought into play to promote the development of rootstock breeding; thirdly, continuing to induce haploid and dihaploid in order to accelerate genome research, functional gene research and breeding of citrus; fourthly, on the basis of allopolyploids and their creation technology, new materials with only small amount of alien genomic components and target traits could be bred by combining with chromosome manipulation techniques.

**Keywords:** citrus; ploidy manipulation; tetraploid; triploid; haploid

植物染色体倍性操作包括倍性增加和消减 (Thorgaard, 1986; Gernand et al., 2005; Zhao et al., 2013; Kreiner et al., 2017), 广泛应用于多倍体研究和育种。通过自然筛选和人工诱导已获得大量植物多倍体材料, 包括多种果树的多倍体类型 (王同坤 等, 2004; Liang et al., 2011; Zhang et al., 2019)。通过倍性消减获得单倍体以及经单倍体加倍获得双单倍体, 在大量农作物中得到应用 (Maluszynski et al., 2003), 但果树单倍体及双单倍体研究及其应用进展较缓慢 (Germanà, 2006, 2008, 2011)。在倍性操作的基础上开展染色体操作, 已在小麦 (*Triticum aestivum*)、棉花 (*Gossypium*)、大白菜 (*Brassica pekinensis*)、黄瓜 (*Cucumis sativus*) 等作物育种上取得了非常可喜的成就, 培育了大批异源附加系、异源代换系、异源易位系 (薛秀庄 等, 1992; 薛秀庄和吉万全, 1993; Multani et al., 1994, 2003; 李炳林 等, 2001; 陈劲枫 等, 2003; 曹清河, 2006; 顾爱侠 等, 2009), 一些高产、抗病、优质的材料还被培育成品种, 如小麦—黑麦 1RS/1BL 易位系具矮秆、高产、抗病等优异性状 (任燕 等, 2006), 在世界范围内广泛应用。染色体操作技术及其创制的材料在作物育种中发挥了巨大作用 (Hechanova et al., 2018; 张爱民 等, 2018; Dang et al., 2019; Li et al., 2019)。

柑橘属 (*Citrus*) 及其近缘植物多数为二倍体 ( $2n = 2x = 18$ ), 自然条件下也有少量多倍体 (梁国鲁, 1990; Hynniewta et al., 2011; Ollitrault & Navarro, 2012)。多年来, 基于利用三倍体培育无籽柑橘新品种等目的, 柑橘多倍体培育及研究得到长足发展, 促进了柑橘育种 (邓秀新和章文才, 1993; 向素琼和梁国鲁, 2003; Grosser & Gmitter, 2011), 单倍体、双单倍体也得到一定程度利用, 满足了部分基础研究的需要 (Aleza et al., 2009a; Wang et al., 2017)。

染色体工程在其他物种中的成就表明, 在倍性操作的基础上进行染色体操作, 可自近缘植物引入优良性状而不太大改变原作物的品质, 是作物育种的有效途径。柑橘的倍性操作具有广阔的应用前景, 但也存在难点, 近年有新的进展。对柑橘倍性操作进行概述, 简要分析其中的难点, 并对后续发展进行展望, 以期在今后倍性操作及利用不同倍性材料进行基础研究和培育新材料提供参考。

# 1 柑橘倍性操作技术研究与应用现状

## 1.1 柑橘四倍体的获得及其应用

### 1.1.1 秋水仙素诱导

秋水仙素处理是诱导植物染色体加倍的主要方法。秋水仙素诱导产生柑橘四倍体的报道较多, 诱导对象有茎尖 (Wakana et al., 2005; Lee et al., 2008)、胚性愈伤组织 (Wu & Mooney, 2002; Zhang et al., 2007)、悬浮细胞 (Dutt et al., 2010)、未发育的胚珠 (Gmitter & Ling, 1991) 和种子 (Surson et al., 2015)。针对不同基因型, 诱导方法和对象有差异, 目前还未见适宜较多基因型且操作较为简易的方法。茎尖诱导可带菌操作, 过程亦较简易, 且诱导获得的多倍体没有童期, 单胚材料和多胚材料均适用, 但诱导率较低, 且易形成嵌合体; 虽部分嵌合体可分离获得纯合四倍体, 却延长了培育周期 (Aleza et al., 2009b)。胚性愈伤组织中存在大量胚性细胞, 诱导率较高 (可达 59%), 再生的四倍体植株可达 42.55% (Zhang et al., 2007), 且组织培养更易于获得纯合四倍体; 诱导处理愈伤悬浮细胞也易获得纯合四倍体, 得率最高达 43.7% (Dutt et al., 2010)。因此, 组织培养结合秋水仙素诱导柑橘四倍体的前景较好。然而, 已知性状的单胚材料均为成年植株, 较难获得愈伤组织, 未知性状的单胚材料诱导获得多倍体的意义不大, 所以这种方法在单胚材料中的应用较难。

### 1.1.2 实生筛选

实生筛选也是目前获得柑橘四倍体的主要方法, 因为 (1) 柑橘部分珠心胚可自发进行染色体加倍 (Aleza et al., 2011; Deng et al., 2019; 周锐 等, 2020), (2) 柑橘  $2n$  配子发生的频率较高且较普遍。

众多柑橘品种 (杂交组合) 通过实生筛选获得了四倍体植株。其中部分品种或杂交组合 (*C. sinensis* × *P. trifoliata*) 获得率可达 20.9%, 但大部分获得率低于 5%, 有一些材料未发现四倍体植株 (Barrett & Hutchison, 1978; 向素琼 等, 2008; Aleza et al., 2011)。天然实生筛选受年份、胚类型等影响, 不同年份间的差异最高可达 10 倍, 这可能与气候等因素有关 (Aleza et al., 2011)。杂种胚四倍体起源与  $2n$  配子有关 (向素琼 等, 2008), 受精卵加倍的概率较  $2n$  配子发生的概率低。筛选  $2n$  花粉授粉以及诱导  $2n$  配子的方法得到较多研究, 并取得了一定进展 (向素琼 等, 2006; 黄桂香 等, 2011)。珠心胚 (或珠心组织) 在种子内的数量较合子胚多, 加倍形成四倍体的概率比杂种胚大 (洪柳 等, 2005; Aleza et al., 2011), 这是导致多胚品种中纯合四倍体较多的主要原因。

### 1.1.3 体细胞融合

细胞融合可克服柑橘常规有性生殖的障碍, 极大程度地扩展了育种材料的来源, 弥补了杂交育种的不足。多年来, 柑橘体细胞融合取得了巨大的成就, 种内、种间以及属间融合获得了大量同源、异源四倍体, 不同的组合超过 500 个, 涉及了大部分柑橘类及近缘种属植物 (郭文武 等, 1998; Guo & Deng, 1998, 2001; Grosser et al., 2000, 2010; Grosser & Gmitter, 2005, 2011; Dambier et al., 2011)。但不同组合的再生率以及真杂种比例有较大差异。在部分植物细胞融合材料中 1 个亲本的染色体片段化甚至消失形成不对称杂种 (Kao, 1977; Wang et al., 2008)。新品种 ‘华柚 2 号’ 即为通过体细胞杂交 (‘国庆 1 号’ 温州蜜橘 + HB 柚) 获得的不对称二倍体胞质杂种, 仅有 HB 柚的核基因组以及温州蜜橘的细胞质, 表现为细胞质雄性不育, 但其他品质与 HB 柚相近 (Guo et al., 2004; 解凯东 等, 2020)。这预示体细胞融合可获得一些带有亲本之一少量基因组成分的材料。刘继红等 (1999)、Liu 和 Deng (2000) 利用 X 射线处理供体、碘乙酸 (IA) 处理受体, 获得了 2 个组合的不对称杂种植株。这类非对称体细胞杂种减少或消除了亲本之一的染色体及细胞质, 仅亲本

之一的部分性状出现在再生植株中 (Bona et al., 2011)。

#### 1.1.4 应用

四倍体柑橘可用作亲本与二倍体杂交获得三倍体无籽柑橘品种 (系)。早在 1958 年无酸柚 (*C. grandis*) 与多籽的四倍体葡萄柚 (*C. paradisi*) 杂交培育成了三倍体品种 ‘Oroblanco’ 和 ‘Melogold’ (Soost & Cameron, 1980, 1985)。迄今, 大量的二倍体与四倍体间倍性杂交获得了三倍体杂种 (邓秀新 等, 1996b; Recupero et al., 2005; Grosser & Gmitter, 2011; Aleza et al., 2012a, 2012b; 宋健坤 等, 2012; 解凯东 等, 2013, 2014; Xie et al., 2019)。但目前推广的优良无籽柑橘品种 (系) 较少。其原因可能是多方面的, 其中有两点较为突出。第一, 童期较长, 短期内不易培育出新品种; 第二, 部分四倍体, 特别是异源四倍体品质较差, 以其为亲本获得的三倍体品质并不理想, 需进一步培育 (Grosser & Gmitter, 2011)。部分四倍体可直接开发为新品种 (Grosser & Gmitter, 2011)。此外, 有人报道应用四倍体花粉授粉有籽柑橘品种可得到无籽或瘪籽果实 (刘可慧 等, 2010), 这可能与四倍体花粉育性较低或受精后杂种败育有关。部分四倍体性状特异 (Dambier et al., 2011; Ruiz et al., 2012), 在控制树形 (Grosser & Gmitter, 2011)、抗病 (Grosser et al., 2003)、耐盐 (Basel et al., 2004; Mouhaya et al., 2008; Saleh et al., 2008; Grosser et al., 2012; Ruiz et al., 2016b; Wei et al., 2020)、耐旱 (Allario et al., 2008, 2009, 2013; Wei et al., 2018)、耐寒 (Oustric et al., 2017)、耐重金属 (Balal et al., 2017)、耐硼和氯过量 (Ruiz et al., 2016a, 2016c) 等方面有一定优势, 有作为优良砧木推广的潜力。

### 1.2 柑橘三倍体的获得及其应用

#### 1.2.1 实生筛选获得三倍体

从二倍体自交、杂交以及自然结实后代中筛选是目前获得三倍体柑橘最简易的方法, 这类三倍体起源于  $2n$  配子, 产生于杂种胚。基因型、年份均会影响三倍体获得率 (Aleza et al., 2010a)。此外, 种子类型对三倍体获得率也有明显影响, 三倍体较多出现在饱满的小种子中 (陈力耕和胡运权, 1981; Toolapong et al., 1996; Chen et al., 2008; Aleza et al., 2010a)。目前, 已有诱导  $2n$  花粉并通过杂交获得柑橘三倍体的报道 (向素琼, 2002; 向素琼 等, 2006; 杨晓伶 等, 2007)。阮氏雪 (2011) 利用秋水仙素诱导福泽柚  $2n$  花粉, 产生频率最高达到了 17.17%, 应用潜力较大。

#### 1.2.2 四倍体与二倍体杂交

四倍体与二倍体杂交可大规模培育三倍体, 并根据基因型实现定向育种 (Grosser & Gmitter, 2011; Aleza et al., 2012a, 2012b)。但四倍体的配子并非全为二倍性 ( $2x$ ), 后代倍性较多样。在部分四倍体与二倍体的杂交中, 三倍体的获得率仅为 10% (宋健坤 等, 2005)。有报道显示, 四倍体作母本时获得三倍体的频率更高 (Aleza et al., 2009b, 2012b)。

#### 1.2.3 胚乳培养及其他方法

柑橘胚乳培养实施较早, 但获得的三倍体较少 (王大元和张进仁, 1978; Gmitter et al., 1990)。在花药培养中也曾发现三倍体 (Germanà et al., 2005), 其机理尚未明确, 可能与自发的细胞融合有关 (党江波 等, 2018)。此外, 还可通过二倍体体细胞—单倍体体细胞融合和二倍体体细胞—配子融合 (Pental et al., 1998) 获得三倍体, 前者已在柑橘中成功, 但后者还未见报道 (Kobayashi et al., 1997; Ollitrault et al., 2000)。

#### 1.2.4 应用

创制三倍体的主要目的是培育优良无籽品种 (系) (Raza et al., 2003; Ollitrault & Navarro, 2012)。目前, 在美国、意大利、日本、西班牙已发布多个四倍体与二倍体杂交的三倍体品种 (邓秀新, 2005;

Aleza et al., 2010b; Cuenca et al., 2010; Ollitrault & Navarro, 2012)。

1.3 柑橘单倍体和双单倍体的获得及其应用

单倍体、双单倍体对基础研究和育种均有重要意义(Forster & Thomas, 2005; Aleza et al., 2009a)。柑橘获得单倍体的方法主要有花药培养、小孢子培养、诱导孤雌生殖和实生筛选(Hidaka et al., 1979; 陈振光 等, 1980; Oiyama & Kobayashi, 1993; Germanà et al., 1994; Toolapong et al., 1996; Germanà & Chiancone, 2001; Yahata et al., 2005a, 2005b; Froelicher et al., 2007; Aleza et al., 2009a; 魏凌鹤, 2015; 王淑明, 2016)。花药培养、诱导孤雌生殖是目前获得柑橘单倍体、双单倍体最有效的方法。双单倍体在柑橘中也有较多报道(Germanà, 2003; Yahata et al., 2005a), 克里曼丁橘、柚类、葡萄柚、甜橙、柠檬、莱檬(*C. aurantifolia*)以及一些杂交柑橘中有单倍体或双单倍体报道, 其中克里曼丁橘的报道(表 1) 较多, 枳和黄皮(*Clausena excavata*)中也有报道, 其中部分单倍体、双单倍体材料停留在愈伤组织和胚性愈伤组织状态。

表 1 已获得的单倍体、双单倍体的柑橘及近缘材料  
Table 1 Citrus and relatives within which haploid, dihaploid obtained

基因型 Genotype	品种(株系) Variety (Line)	获得方法 Obtaining method	材料类型 Type	参考文献 Reference
莱檬 <i>Citrus aurantifolia</i>  克里曼丁橘 <i>C. clementina</i>	墨西哥莱檬 Mexican Lime	花药培养 Anther culture	纯合二倍体胚状体 Homozygous haploid embryoid	魏凌鹤, 2015
	Nules, SRA 63, Monreal Fortune	花药培养 Anther culture	纯合单倍体、二倍体、三倍体、 六倍体 Homozygous haploid, diploid, triploid and hexaploid	Germanà et al., 2005
	Nules	花药培养 Anther culture	单倍体、双单倍体愈伤组织、植 株 Haploid, double haploid callus, plants	Germanà et al., 1994; Germanà et al., 2000
	Nules	三倍体花粉授粉 Triploid pollen pollination	单倍体植株 Haploid plants	Germanà & Chiancone, 2001
	SRA 63	花药培养 Anther culture	单倍体植株 Haploid plants	Germanà & Chiancone, 2003
	SRA 63	辐射花粉授粉 + 胚 拯救 Irradiated pollen pollination + Embryo rescue	单倍体植株(未存活), 胚性愈伤 组织系 Haploid plants (didn't survive), embryonic callus	Ollitrault et al., 1996; Germanà, 2003
	Monreal Rosso, Nules Clemenules	小孢子培养 Microspore culture 辐射花粉授粉 Irradiated pollen pollination	愈伤组织, 单倍体胚状体 Callus, haploid embryoid 单倍体、双单倍体植株 Haploid, double haploid plants	Karasawa et al., 2013; Chiancone et al., 2015 Aleza et al., 2009a
	—	三倍体花粉授粉 Triploid pollen pollination	单倍体 Haploid	Oiyama & Kobayashi, 1993
	—	二倍体与二倍体杂 交 Hybridization between diploid plants	单倍体植株 Haploid plants	Esen & Soost, 1972; Germanà, 2003
	<i>C. clementina</i> × <i>C. tangerina</i>	Ellendale 辐射花粉授粉 Irradiated pollen pollination	单倍体植株 Haploid plants	Froelicher et al., 2007
<i>C. clementina</i> × ( <i>C. paradisi</i> × <i>C. reticulata</i> )	Lee	三倍体花粉授粉 Triploid pollen pollination	单倍体植株 Haploid plants	Oiyama & Kobayashi, 1993

续表 1

基因型 Genotype	品种 (株系) Variety (Line)	获得方法 Obtaining method	材料类型 Type	参考文献 Reference
<i>C. deliciosa</i> × <i>C. paradisi</i>	Mapo	花药培养 Anther culture	单倍体愈伤组织、透明胚状体 Haploid callus, transparent embryoid	Germanà & Recupero, 1997
<i>C. ichangensis</i> × <i>C. reticulata</i>	No. 14	花药培养 Anther culture	单倍体植株 Haploid plants	Deng et al., 1992
柠檬 <i>C. limon</i>	—	花药培养 Anther culture	单倍体愈伤组织、胚状体和叶状体 Haploid callus, embryoid, thallus	Germanà et al., 1991
四季橘 <i>C. microcarpa</i>	—	花药培养 Anther culture	单倍体植株 Haploid plants	陈振光 等, 1980
柚 <i>C. maxima</i>	Banpeiyu	三倍体花粉授粉 Triploid pollen pollination	单倍体植株 Haploid plants	Toolapong et al., 1996
	HB 柚 'Hirado Buntan' pummelo	辐射花粉授粉 Irradiated pollen pollination	单倍体植株 Haploid plants	王淑明, 2016
	—	二倍体实生后代筛选 Selection from progenies seedling of diploid	单倍体植株 Haploid plants	Wang et al., 2017
日本甜夏橙 <i>C. natsudaikai</i>	—	伽玛射线辐射 Gamma ray radiation	单倍体植株 Haploid plants	Karasawa, 1971; Germanà, 2003
葡萄柚 <i>C. paradisi</i>	鸡尾葡萄柚 Cocktail grapefruit	花药培养 Anther culture	单倍体愈伤组织 Haploid callus	王淑明, 2016
<i>C. reticulata</i> × <i>C. sinensis</i>	Fortune	辐射花粉授粉 Irradiated pollen induction	单倍体植株 Haploid plants	Froelicher et al., 2007
<i>C. reticulata</i> × ( <i>C. sinensis</i> × <i>C. reticulata</i> )	Tangor 15-1 × Red Tangerine	二倍体与二倍体杂交 Hybridization between diploid plants	单倍体植株 Haploid plants	Liu et al., 2012
甜橙 <i>C. sinensis</i>	早金甜橙 'Early Gold' sweet orange	花药培养 Anther culture	单倍体系 (愈伤组织、胚状体), 纯合二倍体系 (包括植株) Haploid lines (callus, embryoid), Homozygous diploid lines (including plants)	王淑明, 2016
	红夏橙 'Rohde Red' Valencia	花药培养 Anther culture	单倍体愈伤组织系 Haploid callus lines	王淑明, 2016
	暗柳橙 'An Liu' sweet orange Valencia	花药培养 Anther culture	单倍体愈伤组织系 Haploid callus lines	王淑明, 2016
		花药培养 Anther culture	双单倍体愈伤组织 Double haploid callus	Cao et al., 2011
<i>C. reticulata</i> , <i>C. deliciosa</i> , <i>C. sinensis</i> , <i>C. limon</i> , <i>C. paradisi</i> , Tangelo 'Mapo' ( <i>C. deliciosa</i> × <i>C. paradisi</i> )		花药培养 Anther culture	含单倍体及多倍体的混倍体愈伤组织 Mixoploid callus containing haploid and polyploid	Geraci & Starrantin, 1990
假黄皮 ( <i>Clausena excavata</i> ) (4x)	—	花药培养 Anther culture	二倍体植株 Diploid plants	Froelicher & Ollitrault, 2000
枳 ( <i>Poncirus trifoliata</i> )	—	花药培养 Anther culture	单倍体植株 Haploid plants	Hidaka et al., 1979

单倍体、双单倍体被用于柑橘基因组信息、柑橘的演化研究 (Xu et al., 2013; Wang et al., 2017; Wu et al., 2018)。还可利用单倍体与二倍体体细胞融合获得三倍体 (Kobayashi et al., 1997; Ollitrault et al., 2000)。

## 1.4 其他倍性材料及其应用

除上述倍性材料外, 柑橘中偶然可见其他倍性材料 (Lee, 1988), 如五倍体 (*Citrus iyo* ‘Miyauchiikan’) (Oiyama & Kobayashi, 1991)、六倍体 (‘Bonanza’ 脐橙与黄皮的体细胞杂种、二倍体锦橙 + 种间四倍体体细胞杂种 HR) (Guo & Deng, 1999; 郭文武 等, 2007)、八倍体 (Barrett & Hutchison, 1981) 以及非整倍体 (‘Kiyomi’ 橘橙与金弹种间杂种) (Yasuda et al., 2010)。

# 2 柑橘倍性操作技术应用面临的主要难点

## 2.1 童期较长

果树童期较长, 是育种中的主要难点之一。柑橘及近缘种属的童期为 3 ~ 10 年, 部分品种因生长条件不同可能长达数十年 (陈力耕和胡运权, 1986; 张谷雄和陈建平, 1990), 这对育种极为不利。在柑橘倍性操作中, 大部分材料均需杂交才能获得较为理想的材料。漫长的童期限制了众多优良种质在柑橘育种中的应用。

缩短童期是柑橘育种的重点和难点。植物生长调节剂诱导配合适当的栽培技术可能对于部分材料缩短童期有一定的效果, 现已有通过胚芽嫁接将部分杂交柑橘的童期缩至 2 年的报道 (曹立 等, 2010)。山金柑、早花柠檬、早实枳、早花枳是柑橘短童期的重要资源, 可作为培育短童期材料的亲本进行杂交育种 (李怀福和胡小三, 2005; 张金智, 2010; 马丽丽 等, 2013; 彭爱红 等, 2014; 李恩惠, 2017)。部分亲本童期较长的杂交后代也表现出短童期的现象 (Aleza et al., 2012a), 这说明通过杂交获得童期较短的株系有一定的可能。通过转基因技术缩短童期在多种植物中实现, 但基于安全性考虑在短期内推广的可能性较小。

## 2.2 珠心胚干扰、单胚性多倍体类型较少

珠心胚干扰是指多胚柑橘品种的种子中产生的由珠心组织发育的胚, 会对有性胚的发育和有性胚的发现造成干扰。珠心胚干扰一直是柑橘杂交育种的难题。选择单胚性品种为母本, 后代中真杂种的比例高。柑橘单胚性材料较多, 如柚类、梨橙 (程昌凤 等, 2007)、克里迈丁橘、南丰蜜橘、韦尔金橘、尤力克柠檬、北京柠檬、清见橘橙等, 其中不乏优质单胚性类型 (曹立 等, 2007)。但单胚性的四倍体柑橘较少 (表 2), 严重限制了优质三倍体柑橘品种的大规模培育。单胚性柑橘的后代会发生性状分离, 实生筛选获得的四倍体的基因型与母本不同, 因此单胚性柑橘种子不宜作为四倍体诱导的对象。单胚性柑橘的四倍体诱导只能取成年态组织。在成年态组织无菌材料培养获得成功的前提下 (胡新喜 等, 2008; 谢玉明 等, 2008; 张家银 等, 2008), 利用秋水仙素处理茎尖, 结合茎尖微嫁接则可实现柑橘单胚性四倍体的高频诱导 (Aleza et al., 2009b)。此外, 由部分柑橘品种的单胚性小种子中可获得较大比例的三倍体 (陈力耕和胡运权, 1981), 利用  $2n$  配子直接由这类材料的二倍体自交和杂交中获得三倍体, 可避开单胚材料四倍体较难获得的难题, 沃柑、克里迈丁橘、韦尔金橘等有籽品种可能较易实施, 清见等雄性育性较低但雌性可育的材料或可作为母本选育杂交三倍体。

亲本特异性标记可用于杂种鉴定, 可极大减少由于珠心胚干扰造成的后期杂种筛选的工作量。在一些亲本性状有明显差异的杂交组合中, 父本一方的显性性状可作为杂种筛选的标记 (曹立 等, 2011)。分子标记技术可对杂种进行早期鉴定, 在柑橘中也有应用 (洪棋斌 等, 2003)。柑橘倍性间杂交真杂种也可用分子标记进行鉴定 (解凯东 等, 2013), 以多胚材料为母本的杂交后代中可据此

筛选三倍体杂种（解凯东 等，2014）。多胚四倍体作母本与二倍体杂交，通过早期胚抢救，结合分子标记鉴定，也可获得杂交三倍体。在四倍体与二倍体的杂交中，单进行倍性分析也可鉴定真杂种，即三倍体即为真杂种。

表 2 已获得四倍体的单胚性柑橘材料  
Table 2 Monoembryonic citrus materials within which tetraploid obtained

基因型 Genotype	品种（株系） Variety (Line)	获得方法 Obtaining method	参考文献 Reference
<i>Citrus maxima</i> × <i>C. ichangensis</i>	沙田柚—宜昌橙异源四倍体 ‘Shatian’ pomelo - <i>C. ichangensis</i> allotetraploid	异源二倍体经染色体加倍（秋水仙素诱导） Allodiploid genome doubling (colchicine-induced)	杨星 等，2016
柚 <i>C. maxima</i> 克里曼丁橘 <i>C. clementina</i>	沙田柚 ‘Shatian’ pomelo Clemenules, Fina, Marisol	实生筛选 Seedling selection 秋水仙素/氨磺乐灵诱导茎尖 + 微嫁接 + 扇形分离 Colchicine/oryzalin induction + Micro-grafting + Sectorial chimera segregation	向素琼 等，2008 Aleza et al., 2009b
<i>C. clementina</i> × ( <i>C. unshiu</i> × <i>C. nobilis</i> )	Moncada	秋水仙素/氨磺乐灵诱导茎尖 + 微嫁接 + 扇形分离 Colchicine/oryzalin induction + Micro-grafting + Sectorial chimera segregation	Aleza et al., 2009b
异源四倍体 Allotetraploid	‘Succari’ sweet orange + ‘Hirado Buntan Pink’ pummelo sdg., ‘Nova’ tangelo + ‘Hirado Buntan Pink’ pummelo sdg., ‘Murcott’ tangor + ‘Hirado Buntan Pink’ pummelo sdg., ‘Succari’ sweet orange + ‘Hirado Buntan Pink’ zygotic pummelo	体细胞融合 Somatic cell fusion	Grosser & Gmitter, 2011
<i>C. reticulata</i> × <i>C. sinensis</i>	Umatilla	秋水仙素诱导愈伤组织 Colchicine inducing callus	Wu & Mooney, 2002
柠檬 <i>C. limon</i>	Lisbon	未知 Unknown	Esen et al., 1978

2.3 二倍体水平也可实现无核

柑橘倍性操作的初衷是获得无籽三倍体品种。大多数柑橘类型有单性结实能力，因此在栽培柑橘中大部分二倍体类型中均有无籽品种，可自然产生无籽果实。如柚类自交不亲和，单独栽培即可获得无籽果实；大多脐橙品种天然无籽；其他有籽类型大多也可通过芽变、诱变以及实生选种获得无籽新类型（邓秀新和孙绪华，1996；刘继红 等，1999；洪棋斌，2007）。通过倍性操作获得三倍体无籽类型似乎已经没有必要。

然而，多倍体具器官较大、有效成分含量较高、抗病、抗逆、树形较小等特征（党江波 等，2018，2019），这可能会在三倍体柑橘中体现。三倍体柑橘品种 ‘Safor’ 除无籽外，固/酸比明显高于亲本（Cuenca et al., 2010）；三倍体柑橘品种 ‘Garbí’ 除无籽外，还有晚熟等特点（Aleza et al., 2010b）。这说明虽然有些二倍体无需倍性操作即可产生无籽果实，但倍性操作可对柑橘除无籽外的其他性状进行改良，仍有较好前景。

2.4 其他问题

（1）单倍体/双单倍体得率低。尽管单倍体/双单倍体已在较多材料中获得，但获得柑橘类材料较少，其获得频率依然较低。这较大程度阻碍了柑橘类植物基因组及其演化、柑橘功能基因组等方面的研究，也不利于利用单倍体进行柑橘育种。

（2）低育性。较多优质柑橘材料的育性较低，这些材料经染色体加倍后获得的四倍体的育性

可能会更低, 利用这类低育性的四倍体杂交培育三倍体的难度较大。有研究表明, 部分体细胞杂种的育性偏向于高值亲本, 低育性材料与高育性材料经体细胞融合后可产生高育性的四倍体(邓秀新等, 1992, 1996a)。体细胞融合为利用这些低育性材料的优良性状提供了可能性, 即低育性材料与高育性材料体细胞融合获得四倍体, 四倍体的育性可能会较高, 与其他材料杂交即可将低育性材料的基因组遗传至后代。

### 3 展望

#### 3.1 提高 2n 配子的产生频率

利用 2n 配子可以直接得到三倍体、四倍体。在柑橘中已通过这种方法获得多倍体, 但不同基因型 2n 配子发生频率有较大差异。通过低温、高温、秋水仙素等诱导可提高 2n 配子产生的频率。多年来在许多植物中诱导 2n 配子已获得一定进展(廖玮玮 等, 2011), 其中利用高温热激处理杨树花序, 最高可获得 91.21% 的 2n 花粉以及 87% 的三倍体(Li et al., 2017, 2018; Wang et al., 2017), 这在柑橘中值得借鉴。药剂处理可能会由于不同组织渗透速度不同, 而使处理效果的均一度较差。相比之下, 温度对材料的处理较为均一, 杨树采用人工气候室或离体高温诱导 2n 花粉获得成功, 柑橘也可尝试类似的方法。柚类及其他花器官较集中的材料可尝试利用人工气候室诱导雄配子加倍。

#### 3.2 开发多倍体砧木

由于多倍体柑橘(同源、异源)作砧木有较为优异的表现(Grosser et al., 2003; Grosser & Gmitter, 2011; Balal et al., 2017; Oustric et al., 2017; Wei et al., 2018, 2020), 因此后续可大力发展砧木类多倍体或开发已有的多倍体作为砧木, 以控制树形, 提高抗病性以及耐逆境的耐受能力(党江波等, 2019)。

#### 3.3 单倍体诱变

单倍体诱变育种具有其他倍性材料不可比拟的优势, 其隐性变异可得到表达, 且经加倍后可形成纯合二倍体, 在育种及功能基因组研究中有重要作用(Barro et al., 2001; Szarejko, 2012; Shen et al., 2015)。因此, 在获得更多单倍体的基础上, 可更广泛深入地开展柑橘基因组相关研究; 在现有单倍体及获得更多类型单倍体的基础上, 可建立单倍体突变体库并获得有利的突变体, 以促进柑橘功能基因组研究和育种。

#### 3.4 充分利用近缘种属的优良种质资源

柑橘近缘属种中有较多优良种质, 如宜昌橙和枳属植物耐寒性强, 资阳香橙(*C. junos*)耐盐碱(杨义伶 等, 2011), 大叶金豆(*Fortunella hindsii* var. *chintou*)高抗脚腐病(朱伟生和陈竹生, 1991), 酒饼簕(*Atalantia buxifolia*)及多个枳品种(系)高抗根结线虫病(朱伟生和胡军华, 1997), 罗浮(*F. margarita*)(张戈壁 等, 2004)、部分宜昌橙、香橙、金柑和香橼品种(系)(彭祝春 等, 2010; Deng et al., 2010)对溃疡病的抗性较强, 枳类、酒饼簕、九里香(*Murraya exotica*)、粗柠檬、黄皮(*C. lansium*)、木苹果(*Limonia acidissima*)、假黄皮(*C. excavata*)等对黄龙病有较强的耐受能力(范国成 等, 2009; 林积秀, 2018)。目前已获得了较多上述材料与栽培柑橘的体细胞杂种, 部分材料表现较强的抗(耐)性(甘霖 等, 1995; 郭文武和邓秀新, 2000; Grosser & Chandler, 2000; Vilorio et al., 2004a, 2004b)。这预示经体细胞融合可使栽培柑橘类型获得近缘种属的优良性状。为

减少近缘植物基因组的影响, 可进行栽培类型二倍体体细胞 (2x) 与近缘属种植物配子细胞或单倍体细胞 (x) 间的融合, 获得仅含 1 个外源基因组的异源三倍体材料; 或通过不对称体细胞杂交获得含少量外源染色体的新材料; 进而通过染色体工程在后代中获得带有控制目标性状基因的异源单体附加系、代换系甚至含小片段外源染色体的异源易位系等新种质 (曹清河, 2006; 罗向东, 2006)。这对在保持柑橘品质的前提下改良特定性状较有利。

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