

以雄性不育华柚2号为母本有性杂交 创制柚潜在无核新种质

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摘要:【目的】中国柚地方品种多但多数有种子, 缺乏品质优良且无核的柚新品种。柑橘胞质雄性不育为母系遗传, 以具有该性状的柚为母本与有核柚杂交, 可将雄性不育胞质遗传至有性后代, 有望在二倍体水平实现柚无核新品种选育。【方法】以利用细胞工程技术自主创制的雄性不育胞质杂种柚华柚2号为母本、地方特色有核柚为父本进行有性杂交, 果实成熟期收集种子后, 催芽播种培育杂交后代; 在幼苗阶段依据多倍体形态特征从后代中筛选疑似多倍体并用流式细胞仪对其倍性进行鉴定; 通过亲本重测序开发多态性InDel标记并对杂交后代遗传来源进行鉴定。【结果】以华柚2号为母本, 贡水白柚、雷公柚、菊花芯柚、马家柚和甜柚1号为父本, 配置5个杂交组合; 人工授粉后共获得65个成熟果实, 收集到1163粒种子, 通过催芽播种共获得实生苗781株; 依据多倍体形态特征, 从华柚2号×甜柚1号杂交组合的110株后代中发掘出2株疑似多倍体, 经流式细胞仪倍性鉴定其中1株为四倍体。用6对多态性InDel引物对从华柚2号×贡水白柚和华柚2号×雷公柚随机选取的各44株实生后代进行遗传鉴定, 结果表明, 其均有各自父本的特异条带, 暗示2个杂交组合所有后代均为其相应双亲的有性后代。【结论】以雄性不育胞质杂种柚为母本创制的有性后代, 为二倍体水平的柚无核新品种选育提供了宝贵的种质材料。

关键词: 柑橘; 胞质雄性不育; 无核育种; 遗传鉴定; 华柚2号

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Production of potential seedless pummelo sexual progenies via sexual hybridization using male-sterile cybrid Huayou No. 2 as the female parent

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Abstract: 【Objective】Seedlessness is an important trait in citrus. Numerous pummelo cultivars in China contain seeds, with certain fruits containing over 150 seeds, reducing the edible portion of the fruit and consumer acceptance. Cytoplasmic male sterility (CMS), controlled by maternally inherited mitochondrial genome and nuclear genome, is one of the main reasons for seedlessness in citrus fruits, and it has been utilized to obtain seedless germplasm in citrus. For instance, the Satsuma mandarin is a typical example of the CMS type. In Japan, it was chosen as maternal parent in cross breeding to develop the superior breeding parent Kiyomi tangor, known for its monoembryony and male sterility. Following

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that, Kiyomi tangor was utilized as the female parent to produce various seedless citrus varieties, such as Shiranui, Harumi, Setoka and Harehime through cross breeding. Therefore, the strategy of utilizing citrus varieties with CMS as the maternal parent to cross with seedy varieties can effectively transfer the trait of CMS to the offspring. This approach enables the production of seedless citrus at the diploid level, greatly improving the efficiency of seedless citrus breeding. Huayou No. 2 pummelo is a new seedless citrus variety produced via protoplast fusion with Satsuma mandarin as callus parent and Hira-do Buntan (HB) pummelo as leaf parent, with its mitochondrial genome originated from Satsuma mandarin while the nuclear genome and chloroplast genome inherited from the HB pummelo. Compared to its mesophyll parent HB pummelo, Huayou No. 2 pummelo displayed male sterility and seedlessness (under isolated cultivation), with all other traits staying consistent with HB pummelo, making it ideal for direct cultivation as a new variety. In addition, Huayou No. 2 pummelo carries the CMS cytoplasm from Satsuma mandarin and is a monoembryonic variety. Utilizing it as a female parent for hybridization with other seedy pummelo types holds significant practical value in generating seedless germplasm at the diploid level, as the CMS cytoplasm from Huayou No. 2 pummelo can be transferred to the offspring. With this strategy, in our previous breeding program, Huayou No. 2 pummelo was used as the female parent to cross with Shatian pummelo and Cocktail grapefruit, from which more than 1500 hybrids were obtained. Over 500 hybrids from the cross of Huayou No. 2 pummelo $\times$ Shatian pummelo have exhibited male sterility, providing evidence of the success achieved through this strategy. **【Methods】** Five local pummelo varieties including Gongshuibai, Juhuaxin, Leigong, Majia and Tianyou No. 1 were selected as materials in this study due to their seedy fruits, peel thickness and low edible rate. Five sexual crosses were conducted with Huayou No. 2 pummelo as the female parent and the above five seedy pummelos as the male parents during 2022 to 2023, aiming to produce hybrids that could yield seedless and high-quality fruits with a high edible rate. Controlled pollination was conducted in Huazhong Agricultural University. Following the mature fruits collected, the seeds were extracted and sowed in a growth chamber. After germination, putative polyploids were screened according to the morphological feature and then determined by flow cytometry. By DNA re-sequencing of the parents, InDel (insertion-deletion) markers were mined and used to identify the genetic origin of the plants derived from these crosses. **【Results】** From the five crosses with Huayou No. 2 pummelo as the female parent, and Gongshuibai, Leigong, Juhuaxin, Majia, and Tianyou No. 1 pummelos as the male parents, 65 mature fruits were obtained, from which 1163 mature seeds were extracted and sowed in the chamber. After germination, a total of 781 seedlings were obtained, including 221 seedlings from the cross of Huayou No. 2 $\times$ Gongshuibai pummelo, 238 seedlings from Huayou No. 2 $\times$ Leigong pummelo, 104 seedlings from Huayou No. 2 $\times$ Majia pummelo, 108 seedlings from Huayou No. 2 $\times$ Juhuaxin pummelo and 110 seedlings from Huayou No. 2 $\times$ Tianyou No. 1 pummelo, respectively. Based on the morphological trait screening, we identified two putative polyploids from the seedlings derived from the cross of Huayou No. 2 $\times$ Tianyou No. 1 pummelo and one of them was verified as tetraploid using flow cytometry. Based on DNA re-sequencing of Huayou No. 2, Gongshuibai pummelo and Leigong pummelo, six pairs of polymorphic InDel primers were mined and used for identifying the genetic origin of the offspring derived from Huayou No. 2 $\times$ Gongshuibai pummelo and Huayou No. 2 pummelo $\times$ Leigong pummelo. For each cross, 44 plants were randomly selected and the results showed that all the detected offspring displayed the bands only in their male relative parent, suggesting that all the offsprings of both crosses were the hybrids of their parents. **【Conclusion】** Using a high efficient cell engineering breeding technique in which Huayou No. 2 pummelo was used as the female parent to cross with seedy

pummelos to produce seedless germplasm at the diploid level, hundreds of diploid hybrid and one tetraploid seedlings for potential seedlessness were obtained, providing abundant materials for pummelo seedless breeding at the diploid level. This study also laid a foundation for providing materials for the researches related to male sterility.

Key words: *Citrus*; Cytoplasmic male sterility; Seedless breeding; Genetic identification; Huayou No. 2 pummelo

果实无核是柑橘重要的经济性状,中国大多数地方特色柚为有核品种,部分品种单果种子数超过150粒,降低果实可食率的同时,也影响了消费者的食用体验,综合品质优良且果实无核的柚品种十分缺乏^[1]。细胞质雄性不育(cytoplasmic male sterility, CMS)特性通常表现为母系遗传,由线粒体和细胞核基因组共同决定,也称核质互作不育^[2],是柑橘果实无核的主要原因之一^[3-4]。因此,以具有CMS特性的柑橘为母本与有核品种有性杂交,可利用母系遗传的特性,将细胞质雄性不育特性遗传给后代,能实现在二倍体水平的柑橘无核化改良,提高柑橘无核育种效率。温州蜜柑的果实无核属于典型的CMS类型,日本育种家以温州蜜柑为母本进行有性杂交,选育出了兼具单胚且雄性不育的优良育种亲本清见橘橙^[5],进一步以其为母本,通过杂交育种培育出许多无核品种,如不知火、春见、爱媛、晴姬等^[6]。

华柚2号是华中农业大学以雄性不育的温州蜜柑与有核品种HB柚原生质体融合培育而成的无核柚新品种^[7],线粒体基因组来自温州蜜柑,核基因组和叶绿体基因组均来自HB柚^[4]。与其叶肉亲本HB柚相比,华柚2号除表现雄性不育和果实无核(隔离种植)外,其余性状基本一致,可直接作为鲜食品种发展。华柚2号遗传了温州蜜柑CMS特性,又为单胚品种,以其为母本与其他有核柚有性杂交,在改良中国地方特色柚的有核性状方面具有重要的应用价值,有望在二倍体水平直接培育出果实无核且品质优良的无核柚新品种。前人以华柚2号为母本,沙田柚、鸡尾葡萄柚和温岭高橙为父本进行有性杂交,创制了一批有性后代^[8-9],且华柚2号×沙田柚群体已有500余株开花结果,均表现雄性不育(数据未发表)和果实无核,推测柚细胞核不含有功能性核育性恢复基因,以华柚2号为母本与有核柚有性杂交,能实现二倍体水平的柚无核化改良。贡水白柚、菊花芯柚、雷公柚和马家柚均为中国地方特色良种,果实品质优良,风味浓郁,均有一定的种植面积,经济效

益好,有效带动了当地农业经济发展;甜柚1号是湖南省农业科学学院园艺研究所通过实生选种自主培育的小果型柚品种,风味浓郁。但上述品种的果实均存在有核或多核、果皮厚和可食率低等问题。基于上述问题,笔者以华柚2号为母本,与上述有核柚品种有性杂交创制有性群体;并通过“观根辨叶看油胞”的多倍体发掘技术,从创制的群体中筛选四倍体,创制兼具双亲优良性状且果实无核的二倍体柚新种质,同时有望获得兼具单胚、雄性不育特性的四倍体新种质,为倍性杂交创制柚无核新种质提供核心育种亲本。

1 材料和方法

1.1 试验材料

2022—2023年,以华柚2号(*Citrus grandis* L. Osbeck)为母本,贡水白柚(*C. grandis* L. Osbeck)、雷公柚(*C. grandis* L. Osbeck)、菊花芯柚(*C. grandis* L. Osbeck)、马家柚(*C. grandis* L. Osbeck)和甜柚1号(*C. grandis* L. Osbeck)为父本杂交,创制二倍体有性群体。贡水白柚、菊花芯柚、雷公柚和马家柚花粉分别采自湖北恩施、湖南张家界、湖南郴州和江西上饶;甜柚1号花粉由湖南省农业科学学院园艺研究所提供。华柚2号(原始母树)定植于华中农业大学柑橘育种基地,树龄15 a(年),授粉地点均为华中农业大学。

1.2 人工授粉、实生播种与植株移栽

柑橘花粉制备和人工授粉方法参考解凯东等^[10]的方法。初花期采摘父本处于气球期的含苞待放花朵用于制备花粉,在华柚2号盛花初期进行人工授粉。待果实成熟后,采摘授粉果实剥取种子并催芽播种,催芽播种参考谢善鹏等^[11]的方法。播种后,用塑料薄膜覆盖营养钵保湿,并将播种的营养钵置于生长室[温度(25 ± 1)℃,光照16 h]催芽;待种子萌发和幼苗长至具有6片以上真叶大小时,将幼苗单独移栽至黑色长营养钵,并置于温室保存,幼苗期间

进行正常水肥管理。

1.3 实生后代疑似多倍体筛选和倍性鉴定

利用课题组前期建立的“观根辨叶看油胞”方法^[12]对所有实生后代进行形态观察,筛选疑似多倍体;采集疑似多倍体的叶片,参考谢善鹏等^[11]的方法,用流式细胞仪(Cyflow space, Sysmex, Japan)对其倍性鉴定。

1.4 实生后代的遗传鉴定

杂交后代及其亲本 DNA 提取参照 Cheng 等^[13]的方法。对亲本进行重测序开发可用于后代遗传鉴定的 InDel 分子标记,数据分析参考 Albers 等^[14]的方法,最终从获得的 InDel 变异中开发获得了 6 对多态性 InDel 引物(表 1),引物序列由北京擎科生物科技有限公司合成。PCR 反应体系参考谢善鹏等^[15]的方法,55 ℃退火 30 s,72 ℃延伸 10 min。扩增产物用 2.5% 高分辨率琼脂糖凝胶(CAMBREX, MetaPhor Agarose, USA)于 70 V 电压下电泳 60 min 后,用凝

胶成像仪(Bio-Rad, Universal Hood II, USA)对电泳结果拍照分析。

2 结果与分析

2.1 以华柚 2 号为母本与 5 个有核柚为父本有性杂交创制实生后代 781 株

以华柚 2 号为母本,分别与贡水白柚、菊花芯柚、雷公柚、马家柚和甜柚 1 号等父本进行有性杂交,配置了 5 个杂交组合。由表 2 可知,5 个杂交组合共授粉 408 朵花,坐果 65 个,平均坐果率 15.93%;从 30 个果实共剥取获得种子 1163 粒,其中华柚 2 号×甜柚 1 号组合的单果种子最多,平均 68.3 粒·果⁻¹,华柚 2 号×贡水白柚组合的单果种子最少,平均 20.6 粒·果⁻¹;对所有种子进行催芽播种(图 1),所有组合共获得实生后代 781 株,不同杂交组合的种子萌发率介于 40.3%~94.7%。

2.2 植株形态初选结合倍性鉴定从实生后代发掘出四倍体 1 株

依据柑橘“观根辨叶看油胞”发掘多倍体的方法,对 5 个组合所有实生后代进行多倍体形态初选,从华柚 2 号×甜柚 1 号的实生后代中筛选出 2 株疑似多倍体,其余组合未筛选到疑似多倍体,用流式细胞仪对 2 株疑似多倍体倍性鉴定,其中 1 株为四倍体(图 2);与二倍体植株相比,四倍体主要表现植株变矮、主根短粗、侧根少、叶片厚等特点。

2.3 杂交后代的遗传鉴定

利用亲本重测序,开发获得适用于华柚 2 号×贡水白柚和华柚 2 号×雷公柚组合实生后代分子鉴定的 InDel 引物 6 对(表 1),其中 HG3-1982338、HG3-9173442 和 HG4-24205043 适用于华柚 2 号×

表 1 引物序列信息

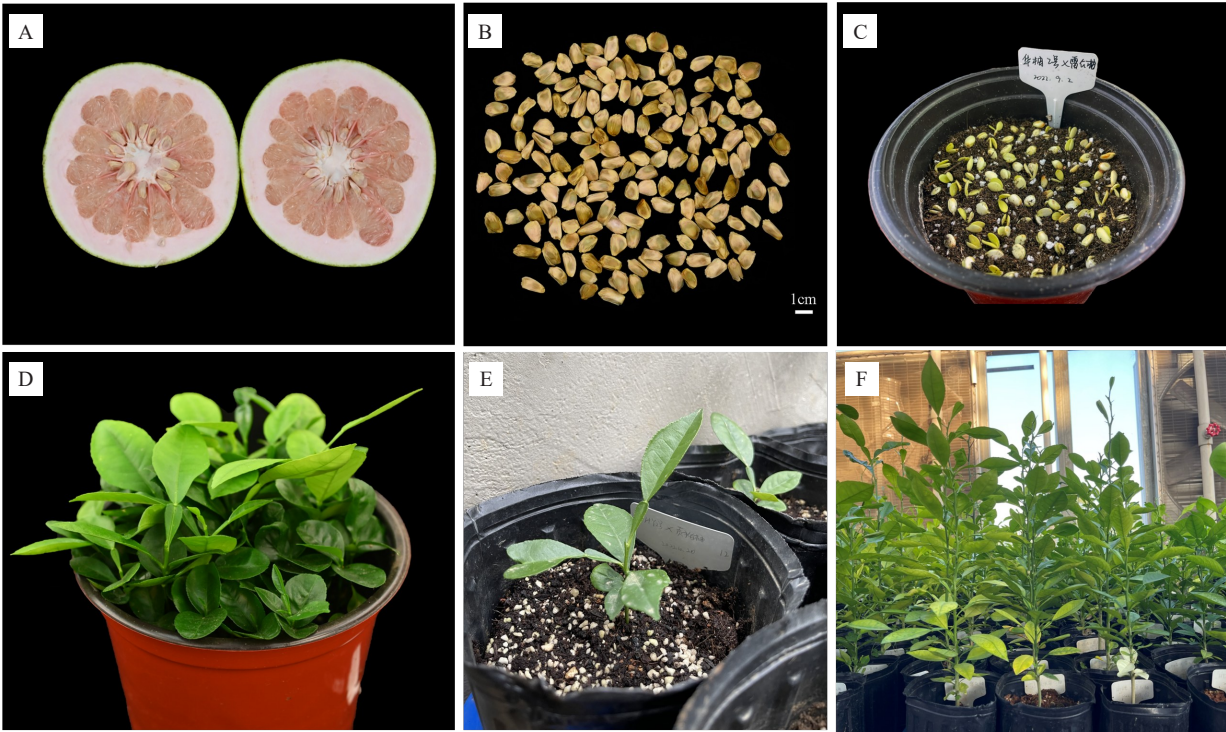
Table 1 Primer Sequences

引物代号 Primer code	引物序列(5'-3') Primer Sequence (5'-3')
HG3-1982338	F:GTGAAATATGATCTGCCGGAAG R:AAAAGTCATCACAGGCAGC
HG3-9173442	F:CGCAGCGCCAATATCACC R:TTTCCTCTAGAAAGTACTTGTATAATTGG
HG4-24205043	F:GTATTCCAAAGTCTTATCTGGGAGG R:TTAGGCCTCGAGTTCTTTATTCA
HL3-25472149	F:TTCTTTTGCAAACCGCCG R:TCGGTGGTTTTCTTTTAACCG
HL3-25864672	F:ATGTTAATTGTTGTTGCAGACTA R:TAGTGCCTGTAGGTTTGGAT
HL4-3919645	F:CCGTACATCCATGGATTAGG R:TGGGGGGGTGACCTG

表 2 以华柚 2 号为母本的 5 个杂交组合有性群体创制情况

Table 2 Progeny production from the five sexual crosses with Huayou No. 2 pummelo as female parent

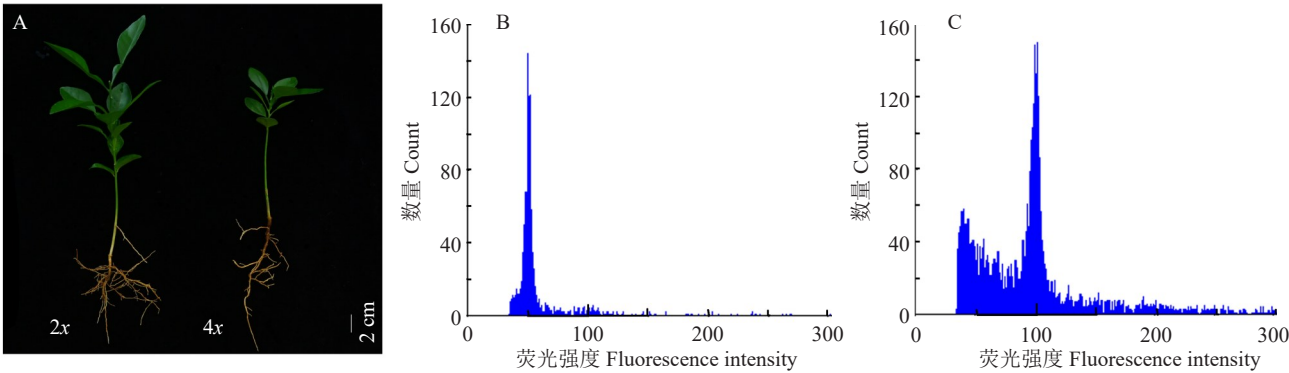
杂交组合 Cross combination	授粉花数 No. of flowers	坐果数 No. of fruits	采果数 No. of fruits collected	种子总数 No. of seeds	单果种子数 No. of seeds per fruit	植株数 No. of plants
华柚 2 号×贡水白柚 Huayou No. 2 pummelo × Gongshuibai pummelo	66	20	14	289	20.6	221
华柚 2 号×雷公柚 Huayou No. 2 pummelo × Leigong pummelo	105	17	6	323	53.8	238
华柚 2 号×甜柚 1 号 Huayou No. 2 pummelo × Tianyou No. 1 pummelo	41	22	4	273	68.3	110
华柚 2 号×马家柚 Huayou No. 2 pummelo × Majia pummelo	136	3	3	164	54.7	104
华柚 2 号×菊花芯柚 Huayou No. 2 pummelo × Juhuaxin pummelo	60	3	3	114	38.0	108
总计/平均 Sum/Average	408	65	30	1163	47.1	781



A. 华柚 2 号 × 雷公柚果实; B. 成熟种子; C. 种子播种于塑料钵; D. 播种后 1 个月实生幼苗; E. 移栽至长塑料钵; F. 移栽后 1 年实生幼苗。
A. The fruit of Huayou No. 2 pummelo × Leigong pummelo; B. Mature seeds; C. Seed germination and sowing; D. One-month old seedlings; E. Transplanted to the pots; F. One-year old seedlings.

图 1 华柚 2 号×雷公柚有性群体创制流程

Fig. 1 Progeny production of Huayou No. 2 pummelo × Leigong pummelo by sexual hybridization



A. 后代植株形态特征; B. 二倍体后代(荧光强度≈50); C. 四倍体后代(荧光强度≈100)。
A. Tetraploid screening based on morphological observation; B. The diploid progeny (Fluorescence intensity ≈ 50); C. Tetraploid progeny (Fluorescence intensity ≈ 100).

图 2 华柚 2 号×甜柚 1 号组合后代倍性鉴定

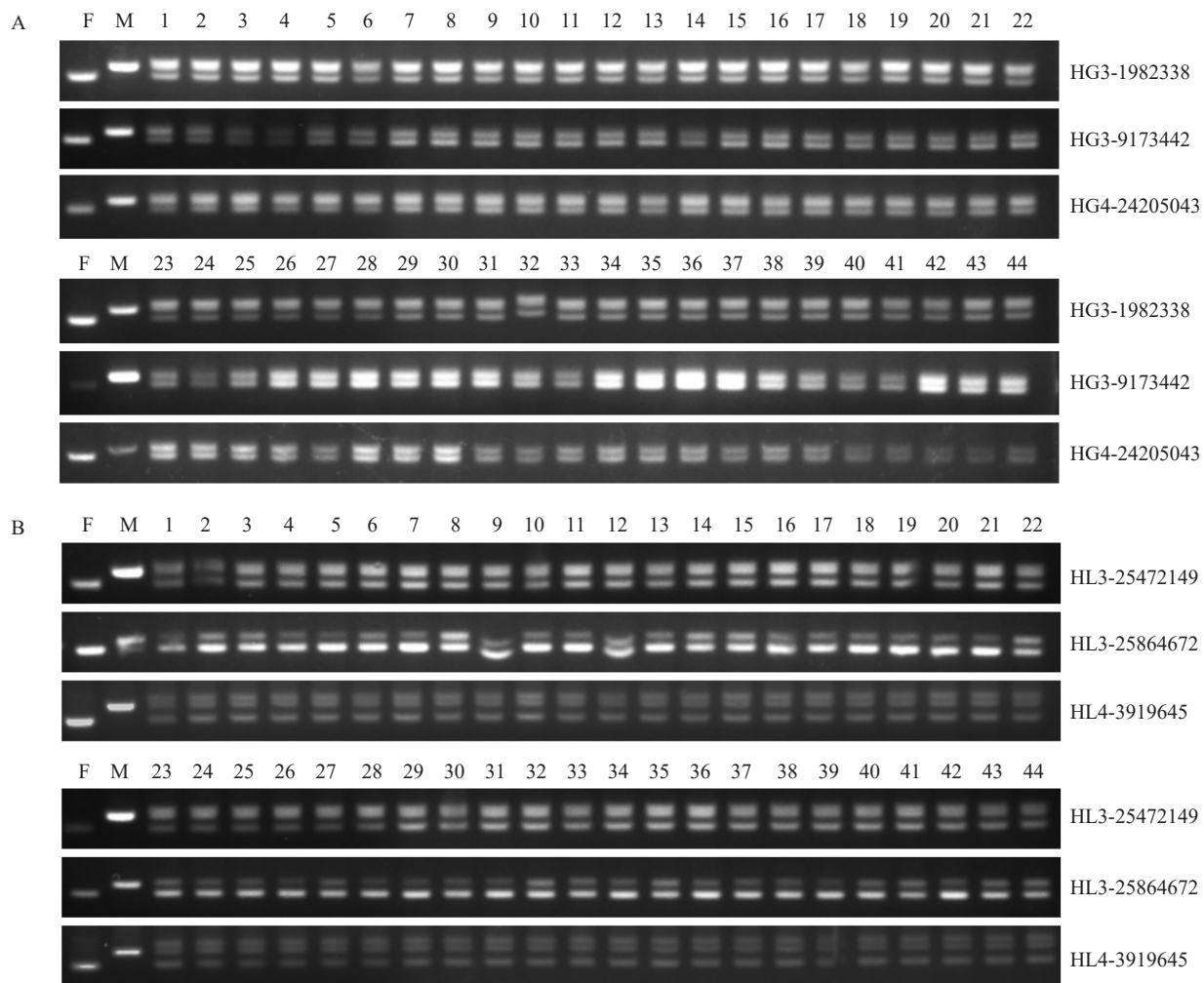
Fig. 2 Ploidy level of the plants from Huayou No. 2 pummelo × Tianyou No. 1 pummelo by flow cytometry

贡水白柚组合; HL3-25472149、HL3-25864672 和 HL4-3919645 适用于华柚 2 号 × 雷公柚组合。分别用上述引物对从 2 个杂交组合各随机筛选的 44 株实生后代进行分子鉴定, 结果表明, 所鉴定的杂交后代均含有父本特异性条带, 表明均为其相应双亲的有

性后代(图 3)。

3 讨 论

笔者利用具有细胞质雄性不育特性的华柚 2 号为母本, 与贡水白柚等 5 个有核柚有性杂交, 以期通



A. 华柚 2 号 × 贡水白柚杂交组合;F. 华柚 2 号;M. 贡水白柚;1~44. 随机选取子代实生苗。B. 华柚 2 号 × 雷公柚杂交组合;F. 华柚 2 号;M. 雷公柚;1~44. 随机选取子代实生苗。

A. The cross of Huayou No. 2 pummelo × Gongshuibai pummelo; F. Huayou No. 2 pummelo; M. Gongshuibai pummelo; 1-44. Randomly selected seedlings. B. The cross of Huayou No. 2 pummelo × Leigong pummelo; F. Huayou No. 2 pummelo; M. Leigong pummelo; 1-44. Randomly selected seedlings.

图 3 杂交后代实生苗的分子鉴定

Fig. 3 Molecular identification of the progenies from the sexual crosses

过将其雄性不育胞质转移至有性后代,创制具有无核潜力的柚新种质,为柑橘二倍体水平的无核育种提供丰富的种质材料。笔者所选的亲本均为二倍体单胚柚品种,理论上其有性后代均应为二倍体,经倍性鉴定从华柚 2 号 × 甜柚 1 号有性后代中发掘到 1 株四倍体幼苗。据前人文献报道可知,四倍体可由 $2n$ 卵细胞与 $2n$ 花粉结合所形成,植物界多数植物材料可产生未减数 $2n$ 配子, $2n$ 配子形成原因主要有无孢子生殖、减数分裂前染色体加倍和纺锤体异常等。向素琼等^[16]通过细胞学观察发现长寿沙田柚 $2n$ 花粉发生频率约为 2%,证实了柑橘中存在未减数的

$2n$ 花粉。Xie 等^[17-18]通过倍性杂交发现柑橘普遍存在 $2n$ 雌配子。因此,本研究二倍体间有性杂交获得的四倍体,可能由 $2n$ 雌配子和 $2n$ 雄配子受精形成。但自然条件下柑橘 $2n$ 配子发生频率较低, $2n$ 雌配子和 $2n$ 雄配子受精形成四倍体的概率更低。此外,柑橘珠心细胞在自然条件下可以自然加倍^[19],二倍体的合子细胞同样可以自发加倍形成四倍体。该四倍体究竟由何种机制产生,需要后续对该四倍体进行基因分析鉴定。四倍体是柑橘倍性杂交创制三倍体无核新种质的核心育种亲本,特别是以单胚四倍体为母本的倍性杂交可省去胚抢救过程,能有效提升

育种效率。但自然界柑橘单胚四倍体资源稀少,限制了该育种途径的应用。笔者在本研究中所选的亲本均为单胚品种,且华柚2号还具有雄性不育特点,从以其为亲本的杂交群体中发掘获得的四倍体可能兼具单胚和雄性不育的特点,在未来的倍性杂交育种中具有重要的应用价值。

自然变异发掘、杂交育种和细胞工程育种是目前实现柑橘无核育种目标的3种主要育种途径^[20]。自然变异相对随机且发生频率低,由此途径获得的无核资源有限。利用杂交育种手段创制无核资源,可有效提高无核育种效率,但柑橘传统杂交育种受多胚性和童期长等影响,杂交育种进程缓慢。通过倍性杂交结合胚挽救技术创制三倍体可直接获得无核种质,但技术难度大且倍性增加对果实品质有一定影响^[9]。因此,将细胞工程技术与杂交育种结合,以单胚性雄性不育胞质杂种为母本与有核品种有性杂交,通过转移雄性不育胞质至有性后代,可实现二倍体水平的无核种质创制,既能保证果实品质又可缩短育种周期,为柑橘无核育种提供了新路径。华中农业大学前期利用细胞工程技术培育了具有细胞质雄性不育特性的胞质杂种华柚2号和华柚3号^[21],均表现雄性不育和果实无核(隔离种植),且种子单胚,以其为核心种源与中国地方特色有核柚品种有性杂交,进而改良柑橘有核性状,具有重要的应用价值。笔者以华柚2号为母本,与贡水白柚等5个有核品种有性杂交,通过创制有性群体以期实现柚有核品种的无核化改良,为培育果实无核、品质优良的柚新品种提供了种质材料。

4 结 论

以具有细胞质雄性不育特性的华柚2号为母本,与贡水白柚等5个有核柚品种有性杂交,获得了一批无核潜力柚新种质,为柑橘二倍体水平的无核改良与雄性不育基础研究提供了种质材料。

参考文献 References:

- [1] 解凯东,彭珺,袁东亚,强瑞瑞,谢善鹏,周锐,夏强明,伍小萌,柯甫忠,刘高平, GROSSER J W, 郭文武. 以本地早橘和椪橘为母本倍性杂交创制柑橘三倍体[J]. 中国农业科学, 2020, 53(23): 4961-4968.
XIE Kaidong, PENG Jun, YUAN Dongya, QIANG Ruirui, XIE Shanpeng, ZHOU Rui, XIA Qiangming, WU Xiaomeng, KE Fuzhi, LIU Gaoping, GROSSER J W, GUO Wenwu. Production of citrus triploids based on interploidy crossing with Bendizao and Man tangerines as female parents[J]. Scientia Agricultura Sinica, 2020, 53(23): 4961-4968.
- [2] DEWEY R E, SELOTE D, GRIFFIN H C, DICKEY A N, JANTZ D, SMITH J J, MATTHIADIS A, STRABLE J, KESTELL C, SMITH W A. Cytoplasmic male sterility and abortive seed traits generated through mitochondrial genome editing coupled with allotopic expression of *atp1* in tobacco[J]. Frontiers in Plant Science, 2023, 14: 1253640.
- [3] GUO W W, PRASAD D, CHENG Y J, SERRANO P, DENG X X, GROSSER J W. Targeted cybridization in citrus: Transfer of *Satsuma cytoplasm* to seedy cultivars for potential seedlessness[J]. Plant Cell Reports, 2004, 22(10): 752-758.
- [4] JIANG N, FENG M Q, CHENG L C, KUANG L H, LI C C, YIN Z P, WANG R, XIE K D, GUO W W, WU X M. Spatiotemporal profiles of gene activity in stamen delineate nucleo-cytoplasmic interaction in a male-sterile somatic cybrid citrus[J]. Horticulture Research, 2023, 10(7): uhad105.
- [5] 郭文武,叶俊丽,邓秀新. 新中国果树科学研究70年:柑橘[J]. 果树学报, 2019, 36(10): 1264-1272.
GUO Wenwu, YE Junli, DENG Xiuxin. Fruit scientific research in new China in the past 70 years: Citrus[J]. Journal of Fruit Science, 2019, 36(10): 1264-1272.
- [6] OMURA M, SHIMADA T. Citrus breeding, genetics and genomics in Japan[J]. Breeding Science, 2016, 66(1): 3-17.
- [7] 解凯东,方燕妮,伍小萌,谢宗周,邓秀新,郭文武. 无核柚新品种‘华柚2号’[J]. 园艺学报, 2020, 47(增刊2): 2946-2947.
XIE Kaidong, FANG Yanni, WU Xiaomeng, XIE Zongzhou, DENG Xiuxin, GUO Wenwu. A new seedless pummelo cultivar ‘Huayou 2’ [J]. Acta Horticulturae Sinica, 2020, 47(Suppl. 2): 2946-2947.
- [8] 夏强明,彭珺,解凯东,伍小萌,徐强,郭文武. 以雄性不育胞质杂种‘华柚2号’为母本创制柚有性群体[J]. 果树学报, 2019, 36(8): 961-967.
XIA Qiangming, PENG Jun, XIE Kaidong, WU Xiaomeng, XU Qiang, GUO Wenwu. Production of sexual hybrids with male sterile somatic cybrid pummelo ‘Huayou No. 2’ as female parent[J]. Journal of Fruit Science, 2019, 36(8): 961-967.
- [9] 张成磊,师小舒,陈昊,谢善鹏,卢鑫,伍小萌,刘高平,郭文武,解凯东. 浙江地方特色品种温岭高橙无核潜力新种质创制及分子鉴定[J]. 果树学报, 2024, 41(4): 590-597.
ZHANG Chenglei, SHI Xiaoshu, CHEN Hao, XIE Shanpeng, LU Xin, WU Xiaomeng, LIU Gaoping, GUO Wenwu, XIE Kaidong. Production and molecular identification of potentially seedless germplasms derived from Wenling-Gaocheng, a citrus local cultivar in Zhejiang province[J]. Journal of Fruit Science, 2024, 41(4): 590-597.
- [10] 解凯东,王晓培,王惠芹,梁武军,谢宗周,郭大勇,伊华林,邓秀新, GROSSER J W, 郭文武. 以柑橘多胚性二倍体母本倍性杂交培育三倍体[J]. 园艺学报, 2014, 41(4): 613-620.

- XIE Kaidong, WANG Xiaopei, WANG Huiqin, LIANG Wujun, XIE Zongzhou, GUO Dayong, YI Hualin, DENG Xiuxin, GROSSER J W, GUO Wenwu. High efficient and extensive production of triploid citrus plants by crossing polyembryonic diploids with tetraploids[J]. *Acta Horticulturae Sinica*, 2014, 41(4): 613-620.
- [11] 谢善鹏, 杨雯惠, 陈昊, 肖公傲, 解凯东, 夏强明, 伍小萌, 郭文武. 国庆1号温州蜜柑珠心胚苗培育及四倍体发掘[J]. *果树学报*, 2023, 40(2): 309-315.
- XIE Shanpeng, YANG Wenhui, CHEN Hao, XIAO Gong'ao, XIE Kaidong, XIA Qiangming, WU Xiaomeng, GUO Wenwu. Production of nucellar seedlings and exploration of tetraploid from Satsuma mandarin Guoqing No. 1[J]. *Journal of Fruit Science*, 2023, 40(2): 309-315.
- [12] 周锐, 解凯东, 王伟, 彭珺, 谢善鹏, 胡益波, 伍小萌, 郭文武. 依据多倍体形态特征快速高效发掘柑橘四倍体[J]. *园艺学报*, 2020, 47(12): 2451-2458.
- ZHOU Rui, XIE Kaidong, WANG Wei, PENG Jun, XIE Shanpeng, HU Yibo, WU Xiaomeng, GUO Wenwu. Efficient identification of tetraploid plants from seedling populations of apomictic citrus genotypes based on morphological characteristics[J]. *Acta Horticulturae Sinica*, 2020, 47(12): 2451-2458.
- [13] CHENG Y J, GUO W W, YI H L, PANG X M, DENG X X. An efficient protocol for genomic DNA extraction from *Citrus* species[J]. *Plant Molecular Biology Reporter*, 2003, 21(2): 177-178.
- [14] ALBERS C A, LUNTER G, MACARTHUR D G, MCVEAN G, OUWEHAND W H, DURBIN R. Dindel: Accurate indel calls from short-read data[J]. *Genome Research*, 2011, 21(6): 961-973.
- [15] 谢善鹏, 解凯东, 夏强明, 周锐, 张成磊, 郑浩, 伍小萌, 郭文武. 柑橘6个地方品种资源四倍体高效发掘及分子鉴定[J]. *果树学报*, 2022, 39(1): 1-9.
- XIE Shanpeng, XIE Kaidong, XIA Qiangming, ZHOU Rui, ZHANG Chenglei, ZHENG Hao, WU Xiaomeng, GUO Wenwu. Efficient exploration and SSR identification of 53 doubled diploid seedlings from six local *Citrus* cultivars and germplasm resources[J]. *Journal of Fruit Science*, 2022, 39(1): 1-9.
- [16] 向素琼, 龚桂枝, 郭启高, 汪卫星, 李春艳, 李晓林, 梁国鲁. 柑橘属 $2n$ 花粉自然发生与沙田柚 $2n$ 花粉诱导研究[J]. *西南农业大学学报(自然科学版)*, 2005, 27(5): 616-620.
- XIANG Suqiong, GONG Guizhi, GUO Qigao, WANG Weixing, LI Chunyan, LI Xiaolin, LIANG Guolu. Spontaneous generation of $2n$ pollen in citrus and induction of $2n$ pollen in *Citrus grandis*[J]. *Journal of Southwest Agricultural University (Natural Science)*, 2005, 27(5): 616-620.
- [17] XIE K D, WANG X P, BISWAS M K, LIANG W J, XU Q, GROSSER J W, GUO W W. $2n$ megagametophyte formed via SDR contributes to tetraploidization in polyembryonic 'Nadorcott' tangor crossed by citrus allotetraploids[J]. *Plant Cell Reports*, 2014, 33(10): 1641-1650.
- [18] XIE K D, XIA Q M, PENG J, WU X M, XIE Z Z, CHEN C L, GUO W W. Mechanism underlying $2n$ male and female gamete formation in lemon via cytological and molecular marker analysis[J]. *Plant Biotechnology Reports*, 2019, 13(2): 141-149.
- [19] 陈昊, 谢善鹏, 解凯东, 肖公傲, 周锐, 伍小萌, 吴群, 邓家锐, 敖义俊, 刘高平, 郭文武. 柑橘13个多胚品种同源四倍体高效发掘与分子鉴定[J]. *果树学报*, 2023, 40(11): 2297-2306.
- CHEN Hao, XIE Shanpeng, XIE Kaidong, XIAO Gongao, ZHOU Rui, WU Xiaomeng, WU Qun, DENG Jiarui, AO Yijun, LIU Gaoping, GUO Wenwu. Efficient exploration and SSR identification of autotetraploids from the seedlings of thirteen apomictic citrus genotypes[J]. *Journal of Fruit Science*, 2023, 40(11): 2297-2306.
- [20] 邓秀新. 中国柑橘育种60年回顾与展望[J]. *园艺学报*, 2022, 49(10): 2063-2074.
- DENG Xiuxin. A review and perspective for citrus breeding in China during the last six decades[J]. *Acta Horticulturae Sinica*, 2022, 49(10): 2063-2074.
- [21] 解凯东, 伍小萌, 方燕妮, 王蓉, 谢宗周, 邓秀新, 郭文武. 无核柚新品种'华柚3号'[J]. *园艺学报*, 2021, 48(增刊2): 2815-2816.
- XIE Kaidong, WU Xiaomeng, FANG Yanni, WANG Rong, XIE Zongzhou, DENG Xiuxin, GUO Wenwu. A new seedless pummelo cultivar 'Huayou 3'[J]. *Acta Horticulturae Sinica*, 2021, 48(Suppl. 2): 2815-2816.