

金柑杂交授粉生物学特性研究

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摘要:【目的】掌握金柑花粉活力与柱头可授性的变化情况, 寻找最佳授粉时期, 探明金柑杂交授粉的生物学特性, 提高杂交成功率。【方法】对23份金柑材料进行胚型的统计。选择不同胚数材料, 采用TTC染色法和联苯胺-过氧化氢法分别对小蕾期、大蕾期、初开期和盛开期的花粉活力和柱头可授性进行研究。对金柑进行去雄不授粉、自交授粉和杂交授粉处理, 比较自交和杂交花粉管的生长情况及3种处理下的坐果率。【结果】罗纹种胚数最少, 适合作母本, 其次是山金柑、金弹和罗浮, 而长寿金柑的胚数最多; 花粉活力和柱头可授性均在4个时期呈先增后减的趋势, 并均在初开期达到了最高活性; 金柑杂交花粉管和部分的自交花粉管均进入了子房; 除宁波金弹和宁波罗纹CS外, 其他5种金柑种质杂交授粉比自交授粉的坐果率更高, 并呈极显著性差异; 母本的柱头活性越高, 坐果率越高, 种内杂交坐果率高于种间, 并呈极显著性差异; 金柑去雄不授粉下仍能得到果实, 具有单性结实的特性。【结论】金柑杂交中应选择单胚型母本材料; 金柑属于雌雄同熟, 采集花粉最佳时期和授粉最佳时期均为初开期; 金柑杂交亲和性比自交亲和性更强, 有利于金柑的杂交育种。

关键词:金柑; 花粉活力; 柱头可授性; 花粉管发育; 种间杂交

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Study on the biological characteristics of cross-pollination in kumquat

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Abstract:【Objective】Pollen viability and stigma pollinability are important indicators for assessing the quality of parental gametes. Understanding the changing pattern of kumquat pollen viability and stigma pollination rate and finding the best pollination period can effectively improve the quality and success rate of hybrid breeding. Simultaneously, the growth of kumquat pollen tubes under self-pollination and heterogametic pollination conditions was studied to find out the biological characteristics and mechanism of kumquat heterogametic pollination to provide a theoretical basis for kumquat promiscuity. By studying the fruiting rate under different treatment conditions, we can provide some practical basis for kumquat promiscuity.【Methods】Embryo types of 23 *Fortunella* germplasm resources were statistically analyzed. Materials with different embryo numbers were selected to analyze pollen viability and stigma receptivity at four different stages (Small bud stage, Big bud stage, Early fluorescence stage and Full bloom stage) by using TTC staining and benzidine-peroxidase methods, respectively. Subsequently, three treatments, including emasculation without pollination, artificial self-pollination, and hybrid pollination were conducted. The growth of pollen tubes was compared under both artificial self-pollination and hybrid pollination. Finally, fruit setting rates after three treatments were statistically analyzed and compared.【Results】*Fortunella japonica* (Thunb) Swingle had the fewest embryos, making them suitable as female parents, followed by *F. hindsii* (Champ) Swingle, *F. crassifolia* Swingle and *F. margarita* (Lour.) Swingle. *F. obovata* Tanaka had the most embryos. Pollen viability of all tested Kumquat materials at all four stages followed the same trend of “increasing first and then decreasing” and

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the peak reached at the early florescence stage. The highest pollen viability was found in the Lanshanjingan (*F. crassifolia* Swingle), which was significantly different from other species, and Liuyangjingan CQ (*F. crassifolia* Swingle) had the lowest pollen viability. Stigma receptivity followed an increasing-decreasing trend at all four stages, with maximum receptivity observed at the early florescence stage. Lanshanjingan exhibited the highest pistil receptivity, while Liuyangjingan CQ showed the lowest. There were no significant differences in pistil receptivity among other kumquat varieties, and therefore they can all serve as female parents for hybridization. Analysis of pollen activity and stigma receptivity revealed that kumquats exhibited synchronous maturation of male and female reproductive organs, reaching peak values at the early florescence stage. Pollen tubes of both self-pollination and hybridization treatments began germination approximately 2 hours after pollination. Hybrid pollen tubes showed a faster elongation rate compared with self-pollination pollen tubes. After 12 to 24 hours of pollination, most hybrid pollen tubes reached the base of the style as a result of fast elongation, whereas some self-pollination pollen tubes had already ceased elongation at 2/3 of the style length. After 48 hours of pollination, all hybrid pollen tubes and a few self-pollination pollen tubes penetrated the base of the style and entered the ovary. Under the condition of emasculation without pollination, all 7 kumquat germplasm obtained fruits, and Lanshanjingan had the highest fruiting rate and showed significant difference from other kumquat germplasm. The kumquat flowers can develop into fruits without pollination, demonstrating the characteristic of parthenocarpy. Under conditions of artificial self-pollination, Lanshanjingan had the highest fruit setting rate, showing significant differences from other kumquat germplasms. Under the condition of hybridization pollination treatment, the fruiting rate of Liuyangjingan CQ was the highest, followed by Lanshanjingan. However, there was no significant difference in fruit set rate between these two kumquat varieties, but there was a significant difference between them and among the other 5 kumquat varieties. Except for Ningbolouwen CS [*F. japonica* (Thunb) Swingle], the fruit setting rates of the other 6 kumquat from cross-pollination treatments were higher than those from artificial self-pollination; among the 7 kumquat varieties, only Ningbojindan (*F. crassifolia* Swingle) showed no significant difference in fruit setting rates under both artificial self-pollination and cross-pollination treatments. The difference in fruit set rate indicated that the cross-compatibility of kumquats was stronger than self-compatibility, which was more conducive to the hybrid breeding of kumquats. With the same hybrid paternal parent, the higher the stigma pollinability of the female parent, the higher the fruit setting rate. The fruit setting rate of intraspecific hybridization was higher than that of interspecific hybridization, and there was a significant difference. **【Conclusion】**The female parent with single embryo should be selected in kumquat hybridization; kumquat belonged to hermaphroditism, and the best time for pollen collection and pollination was at the early florescence stage; kumquat cross-compatibility was stronger than self-compatibility, which was beneficial to kumquat cross breeding.

Key words: Kumquat; Pollen viability; Stigma pollinability; Pollen tube development; Interspecific hybridization

金柑属(*Fortunella* Swingle, 1915)植物, 别名金橘等, 原产中国, 为芸香科(Rutaceae)柑橘亚科(Aurantioideae)柑橘族(Citreae)柑橘亚族(Citrinae)的一类植物。主要包括金豆(*F. hindsii* Swingle)、罗纹(*F. japonica* Swingle)、长叶金柑(*F. polyandra* Tanaka)、罗浮(*F. margarita* Swingle)4个种和长寿

金柑(*F. obovata* Tanaka)、金弹(*F. crassifolia* Swingle)2个杂交种^[1], 在广西融安、阳朔、湖南浏阳和蓝山等地广泛种植^[2-3], 食用价值和药用价值极高^[4-9], 国内外市场竞争力强^[10-13], 具有广阔的发展前景。

目前, 果树育种主要采用杂交育种和芽变育种两个途径。据不完全统计, 在中国的苹果、桃及梨等

11种果树中,约有63.9%的新品种是通过杂交育种获得的^[14]。然而,柑橘类植物的育种仍以芽变育种为主,通过杂种选育得到的新品种仅占6.3%^[15]。杂交育种可以从父母本获得优良性状,能够满足人们对培育果树新品种的需求,因此传统的人工杂交育种技术在培育新品种方面仍具有重要地位^[16]。

在植物的杂交育种中,花粉是遗传物质传递的中间媒介^[17],掌握花粉的生物学特性可以有效提高授粉成功率,对选择合适的杂交亲本意义重大^[18],而柱头的可授性则直接影响到受精能否继续进行^[19]。因此,研究花粉柱头活性,选择优良的杂交亲本,可以有效提高结实率,有助于培育杂交新品种^[20]。近年来,学者们对花粉活力和柱头可授性等的研究越发深入,为杂交育种提供了坚实的理论支持;Liu等^[21]以拟南芥为研究对象,揭示了花粉-柱头保守的识别机制,这对克服远缘杂交障碍、获得优良性状的品种具有重要意义;朱江华等^[22]通过对比研究6个蓝莓品种的花粉量、花粉活力及花粉萌发率等花粉

特性,发现花粉量大、萌发率高、花粉活力好的品种在授粉过程中表现出特殊的优异性。

笔者在本研究中通过比较不同时期的花粉活力与柱头可授性,确定最佳授粉时期。对金柑进行去雄不授粉、自交授粉和杂交授粉三种处理并比较自交授粉和杂交授粉的花粉管的生长情况及三种处理下的坐果率,初步探明金柑杂交授粉的生物学特性,为金柑杂交育种提供一定的理论依据。

1 材料和方法

1.1 试验材料

试验材料采自湖南农业大学金山金柑资源圃,其中,金弹种15份材料,罗纹种3份材料,罗浮种2份材料,山金柑种2份材料和长寿金柑种1份材料,共计23份材料(表1)。

1.2 试验方法

试验于2020年6—8月在金山基地进行。并将金柑开花动态分为以下4个时期^[23],每个时期对应

表1 试验供试材料

Table 1 Test materials

种质 Germplasm	来源地 Origin	类型 Type	种质 Germplasm	来源地 Origin	类型 Type
宁波金弹 Ningbojindan	浙江,宁波 Ningbo, Zhejiang	金弹 <i>F. crassifolia</i> Swingle	蓝山金柑 Lanshanjingan	湖南,蓝山 Lanshan, Hunan	金弹 <i>F. crassifolia</i> Swingle
宁波金弹 CQ Ningbojindan CQ	浙江,宁波 Ningbo, Zhejiang	金弹 <i>F. crassifolia</i> Swingle	花叶金柑 Huayejingan	湖南,浏阳 Liuyang, Hunan	金弹 <i>F. crassifolia</i> Swingle
浏阳金柑 Liuyangjingan	湖南,浏阳 Liuyang, Hunan	金弹 <i>F. crassifolia</i> Swingle	融安脆蜜 Rong'ancuimi	广西,融安 Rong'an, Guangxi	金弹 <i>F. crassifolia</i> Swingle
浏阳金柑 CQ Liuyangjingan CQ	湖南,浏阳 Liuyang, Hunan	金弹 <i>F. crassifolia</i> Swingle	宁波罗纹 Ningboluowen	浙江,宁波 Ningbo, Zhejiang	罗纹 <i>F. japonica</i> (Thunb) Swingle
浏阳金柑 CS Liuyangjingan CS	湖南,浏阳 Liuyang, Hunan	金弹 <i>F. crassifolia</i> Swingle	宁波罗纹 CQ Ningboluowen CQ	浙江,宁波 Ningbo, Zhejiang	罗纹 <i>F. japonica</i> (Thunb) Swingle
温州金弹 Wenzhoujindan	浙江,温州 Wenzhou, Zhejiang	金弹 <i>F. crassifolia</i> Swingle	宁波罗纹 CS Ningboluowen CS	浙江,宁波 Ningbo, Zhejiang	罗纹 <i>F. japonica</i> (Thunb) Swingle
温州金弹 CS Wenzhoujindan CS	浙江,温州 Wenzhou, Zhejiang	金弹 <i>F. crassifolia</i> Swingle	福建大果罗浮 Fujiandaguoluofu	福建,永春 Yongchun, Fujian	罗浮 <i>F. margarita</i> (Lour.) Swingle
富圆金柑 Fuyuanjingan	广西,融安 Rong'an, Guangxi	金弹 <i>F. crassifolia</i> Swingle	牛奶金柑 Niunaijingan	广西,阳朔 Yangshuo, Guangxi	罗浮 <i>F. margarita</i> (Lour.) Swingle
滑皮金柑 Huapijingan	广西,阳朔 Yangshuo, Guangxi	金弹 <i>F. crassifolia</i> Swingle	小果金豆 Xiaoguojindou	湖南,郴州 Chenzhou, Hunan	山金柑 <i>F. hindsii</i> (Champ) Swingle
宁波金弹 CS Ningbojindan CS	浙江,宁波 Ningbo, Zhejiang	金弹 <i>F. crassifolia</i> Swingle	大果金豆 Daguojindou	湖南,郴州 Chenzhou, Hunan	山金柑 <i>F. hindsii</i> (Champ) Swingle
广西金弹 Guangxijindan	广西,阳朔 Yangshuo, Guangxi	金弹 <i>F. crassifolia</i> Swingle	公孙橘 Gongsunju	云南,澄江 Chengjiang, Yunnan	长寿金柑 <i>F. margarita</i> (Lour.) Swingle
遂川金弹 Suichuanjindan	江西,遂川 Suichuan, Jiangxi	金弹 <i>F. crassifolia</i> Swingle			

注:CQ 表示引种于中国农业科学院柑橘研究所(重庆);CS 表示引种于湖南省园艺研究所(长沙)。

Note: CQ means introduced from Citrus Research Institute of Chinese Academy of Agricultural Sciences (In Chongqing); CS means introduced in Hunan Horticultural Research Institute (In Changsha).

的花蕾形态如图1。

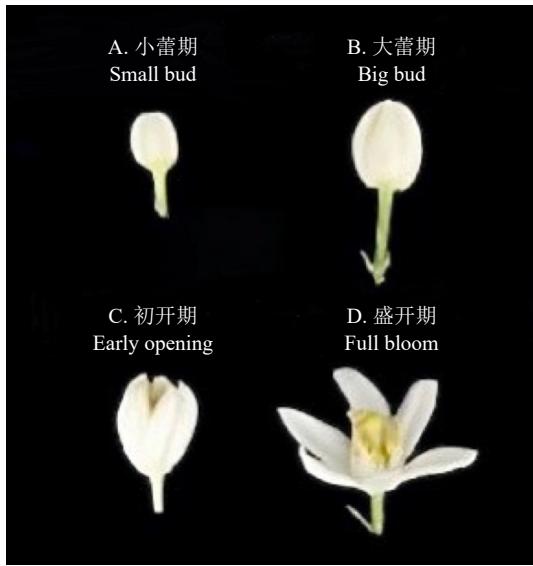


图1 金柑花蕾的四种形态

Fig. 1 Four forms of *Fortunella* flower buds

1.2.1 自然授粉果实胚数统计 在自然授粉结实条件下,从23份种质资源成熟果实中取出种子,去除种皮,将子叶分开观察胚数,每种材料至少5个果实,计算种子数与胚数的平均值。按照胚数从少到多分别选择罗纹种的宁波罗纹、宁波罗纹CQ和宁波罗纹CS,金弹种的宁波金弹、宁波金弹CQ、浏阳金柑CQ、温州金弹CS、蓝山金柑和浏阳金柑,罗浮种的牛奶金柑用于后续试验。

1.2.2 花粉活力的测定 参照0.5%TTC染色法^[24]并略作修改,分别在金柑花蕾的小蕾期、大蕾期、初开期和盛开期4个时期,各采集10个花蕾样本。取花药置于载玻片上,滴加1~2滴0.5%TTC溶液(2,3,5-uiphenyl tetrazolium chloride),置于湿润的培养皿中,室温下静置30~45 min,在显微镜视野中随机选择5个不同的位置,统计总花粉粒数和变色花粉粒数(包括红色和粉色)。按照公式1计算花粉活力。

$$\text{花粉活力} / \% = \frac{\text{变色花粉数}}{\text{观察花粉总数}} \times 100。 \quad (1)$$

1.2.3 柱头活性的测定 参照联苯胺-过氧化氢法^[25],分别在金柑花蕾的小蕾期、大蕾期、初开期和盛开期4个时期,各采集10个花蕾样本,单独取柱头于卡诺式固定液中固定,取出柱头,浸入滴有联苯胺-过氧化氢溶液的凹面载玻片中,室温静置10 min后在显微镜下观察柱头周围的气泡产生及颜色变化情况。柱头可授性检测标准参照焦雪辉等^[24]的方法

(表2)。

表2 柱头可授性检测标准

Table 2 Acceptability test standard of stigma

颜色变化 Color change	气泡产生量 Bubble production	柱头可授性 Stigma acceptability	分值 Score
变蓝 Turn blue	大量 A lot	极强 Extremely strong	6
变蓝 Turn blue	较多 More	很强 Very strong	5
变蓝 Turn blue	较少 Less	强 Strong	4
不变 Unchanged	大量 A lot	较强 A little strong	3
不变 Unchanged	较多 More	较弱 Weak	2
不变 Unchanged	较少 Less	极弱 Extremely weak	1
不变 Unchanged	无或极少 No or few	无 None	0

1.2.4 花粉的采集与授粉 基于花粉活力与柱头可授性的试验结果,选定宁波金弹、浏阳金柑CQ、宁波罗纹、宁波罗纹CQ、蓝山金柑、宁波罗纹CS、牛奶金柑及温州金弹CS,共计8种材料为杂交亲本。自交及杂交组合情况详见表3。

表3 金柑自交及杂交组合

Table 3 Self-pollination and Cross-breeding Combinations of Kumquats

自交组合 Self-cross combination	杂交组合 Hybridization combination
宁波金弹 Ningbojindan	宁波金弹(♀)×温州金弹CS(♂) Ningbojindan (♀)×Wenzhoujindan CS (♂)
浏阳金柑 CQ Liuyangjingan CQ	浏阳金柑CQ(♀)×蓝山金柑(♂) Liuyangjingan CQ (♀)×Lanshanjingan (♂)
宁波罗纹 Ningboliuwen	宁波罗纹(♀)×温州金弹CS(♂) Ningboliuwen (♀)×Wenzhoujindan CS (♂)
宁波罗纹 CQ Ningboliuwen CQ	宁波罗纹CQ(♀)×温州金弹CS(♂) Ningboliuwen CQ (♀)×WenzhoujindanCS (♂)
蓝山金柑 Lanshanjingan	蓝山金柑(♀)×浏阳金柑CQ(♂) Lanshanjingan (♀)×Liuyangjingan CQ (♂)
宁波罗纹 CS Ningboliuwen CS	宁波罗纹CS(♀)×蓝山金柑(♂) Ningboliuwen CS (♀)×Lanshanjingan (♂)
牛奶金柑 Niunaijingan	牛奶金柑(♀)×浏阳金柑CQ(♂) Niunaijingan (♀)×Liuyangjingan CQ (♂)

每天早晨07:00—08:30采集父本花蕾接近初开期之前的雄蕊。取花药并在干燥器中干燥至其花粉散落,4℃冰箱中保存备用。

每天上午07:00—09:00进行授粉。

(1)去雄不授粉:去除母本花蕾接近初开期之前的雄蕊,套上硫酸纸袋并记录去雄时间,3 d后取下

硫酸袋,10 d后统计坐果率。

(2)自交授粉:授粉前1 d下午18:00,去除目标材料花瓣和雄蕊,套上硫酸袋。第2天早上07:00,用毛刷进行人工授粉。授粉时,用毛笔刷轻轻地将花粉刷在柱头上,直到柱头表面明显观察到黄色花粉,确保授粉成功。每朵花连续3 d在相同时间授粉3次,从第3天授粉完成后开始计算,3 d后去袋,10 d后统计坐果率。

(3)杂交授粉:按照表3的杂交组合,将处理好的父本花粉涂抹在选定的母本柱头上,其他操作与自交授粉相同。

1.2.5 花粉管生长观察 在大蕾期,按照表3所示的自交与杂交组合分别进行授粉处理,授粉方法与1.2.4节相同,在授粉结束后的2、4、8、12、24、48 h分别取10个花柱从子房基部切除,于卡诺式固定液中固定12~24 h。之后分别经过70%乙醇、50%乙醇、30%乙醇及蒸馏水冲洗后,放于2 mol·L⁻¹的NaOH溶液中软化8~12 h。最后在0.1%水溶性苯胺蓝中染色12~24 h,用解剖刀切开柱头并压片,在荧光显微镜下观察花粉管生长情况并拍照。

1.2.6 坐果率的统计 统计各种处理下的授粉花朵

数,并在授粉完成后的第10天统计坐果数,按照公式2计算坐果率。

$$\text{坐果率}/\% = \frac{\text{坐果数}}{\text{授粉花朵数}} \times 100。 \quad (2)$$

1.2.7 数据处理与分析 本试验数据通过Excel进行记录、统计,采用SPSS 26.0进行方差分析,用Origin 2018进行图表绘制。

2 结果与分析

2.1 金柑种质资源胚数分析

23份种质的种子数及胚数统计结果如表4,罗纹种胚数均为1个,为单胚型种质,最适合做母本;其次为山金柑、金弹和罗浮,胚数最多的为长寿金柑。

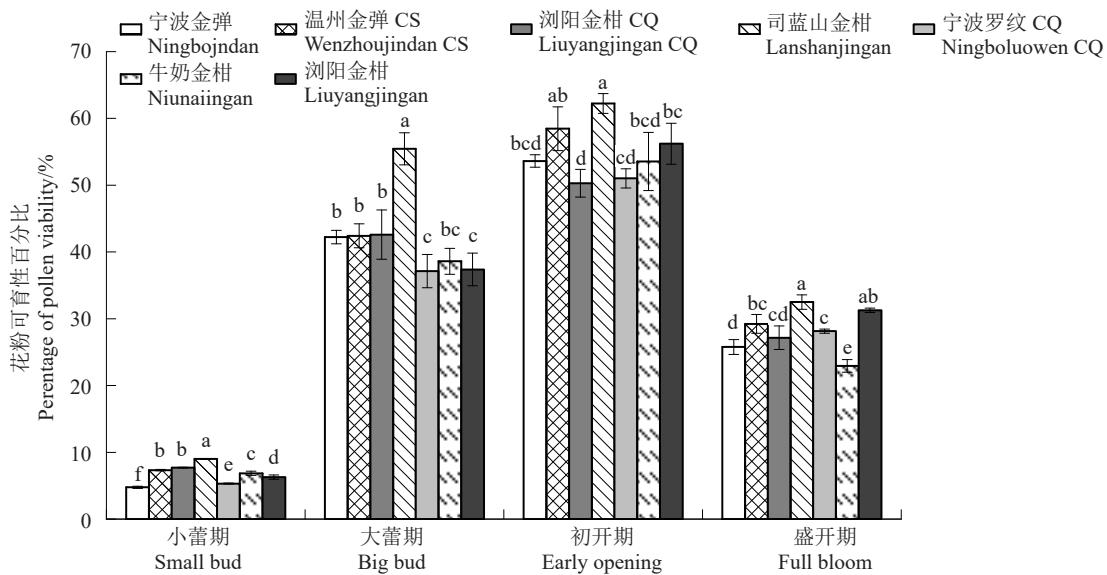
2.2 金柑不同时期花粉活力比较

不同金柑花蕾在4个时期的花粉活力均呈现先升后降的趋势,并在初开期达到了最强水平(图2)。相同时期下,蓝山金柑的花粉活力最强,与除温州金弹CS以外的其他金柑种质存在显著差异($p < 0.05$)。在大蕾期,宁波金弹、温州金弹CS和浏阳金柑CQ间不存在显著性差异($p > 0.05$),且该3种金柑与蓝山金柑、宁波罗纹CQ和浏阳金柑呈显著性

表4 单果种子数及胚数统计

Table 4 Statistics of the number of seeds and embryos of a single fruit

种质 Germplasm	种子数 Number of seeds	胚数 Number of embryos	种质 Germplasm	种子数 Number of seeds	胚数 Number of embryos
宁波金弹 Ningbojindan	4.92	2.60	蓝山金柑 Lanshanjingan	8.67	1.23
宁波金弹 CQ Ningbojindan CQ	4.82	2.35	花叶金柑 Huayejingan	5.57	1.10
浏阳金柑 Liuyangjingan	7.85	2.54	融安脆蜜 Rong'ancuimi	5.13	2.21
浏阳金柑 CQ Liuyangjingan CQ	7.82	2.57	宁波罗纹 Ningboliuwen	6.51	1.00
浏阳金柑 CS Liuyangjingan CS	6.83	1.52	宁波罗纹 CQ Ningboliuwen CQ	4.47	1.00
温州金弹 Wenzhoujindan	4.82	2.47	宁波罗纹 CS Ningboliuwen CS	6.31	1.00
温州金弹 CS Wenzhoujindan CS	6.41	2.64	福建大果罗浮 Fujiandaguoluofu	3.45	1.21
富圆金柑 Fuyuanjingan	4.05	2.73	牛奶金柑 Niunaijingan	5.02	3.31
滑皮金柑 Huapijingan	3.07	3.30	小果金豆 Xiaoguojindou	2.35	1.75
宁波金弹 CS Ningbojindan CS	4.18	2.53	大果金豆 Daguojindou	2.57	1.31
广西金弹 Guangxijindan	5.64	1.75	公孙橘 Gongsunju	17.85	4.33
遂川金弹 Suichuanjindan	5.54	2.87			



误差线为标准误,不同小写字母表示不同类型材料在相同时期花粉活力的差异显著($p<0.05$)。下同。

The error bars are standard errors, and different small letters indicate significant differences in pollen viability of different types of materials at the same time ($p<0.05$). The same below.

图2 金柑不同时期花粉活力测定

Fig. 2 Determination of pollen viability in kumquat at different times

差异($p<0.05$)。

杂交中为避免串粉现象,选择接近初开期的大蕾期为采集花粉的时期。在大蕾期,金柑花粉活力从高到低分别为蓝山金柑>浏阳金柑CQ>温州金弹CS>宁波金弹>牛奶金柑>宁波罗纹CQ>浏阳金柑。选择花粉活力较强的种质作为杂交授粉的父本,即蓝山金柑、浏阳金柑CQ、温州金弹CS。

2.3 金柑不同时期柱头可授性比较

不同金柑花蕾在4个时期的柱头可授性均呈现

先增强后减弱的趋势,并在初开期达到了最高水平(图3)。在大蕾期时,蓝山金柑的柱头可授性高于其他金柑并存在显著性差异($p<0.05$),除蓝山金柑外的其他7种金柑间不存在显著性差异($p>0.05$)。

柱头可授性的强弱会直接影响花粉在柱头上的萌发概率,柱头可授性越强,接受花粉的能力越强,从而更容易完成受精过程。通过对金柑4个时期柱头活性的检测,发现金柑在初开期普遍表现出最强的柱头可授性。所测材料均适用作人工杂交授粉的

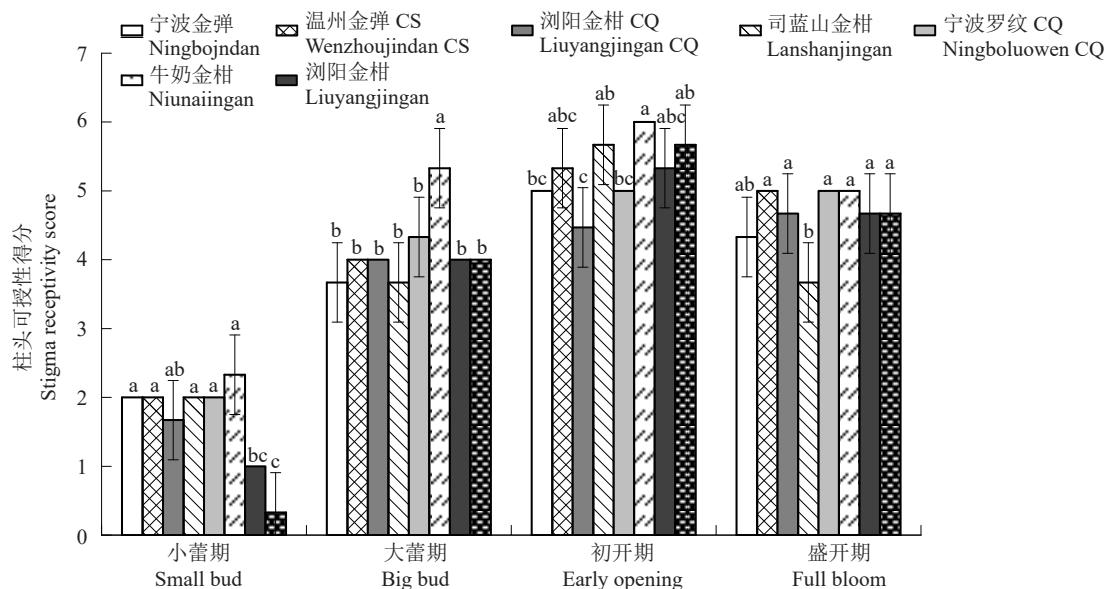


图3 金柑不同时期柱头可授性测定

Fig. 3 Determination of stigma pollinability in kumquat at different times

母本材料。然而,为了避免串粉现象,应在接近初开期之前去除雄蕊并授粉。

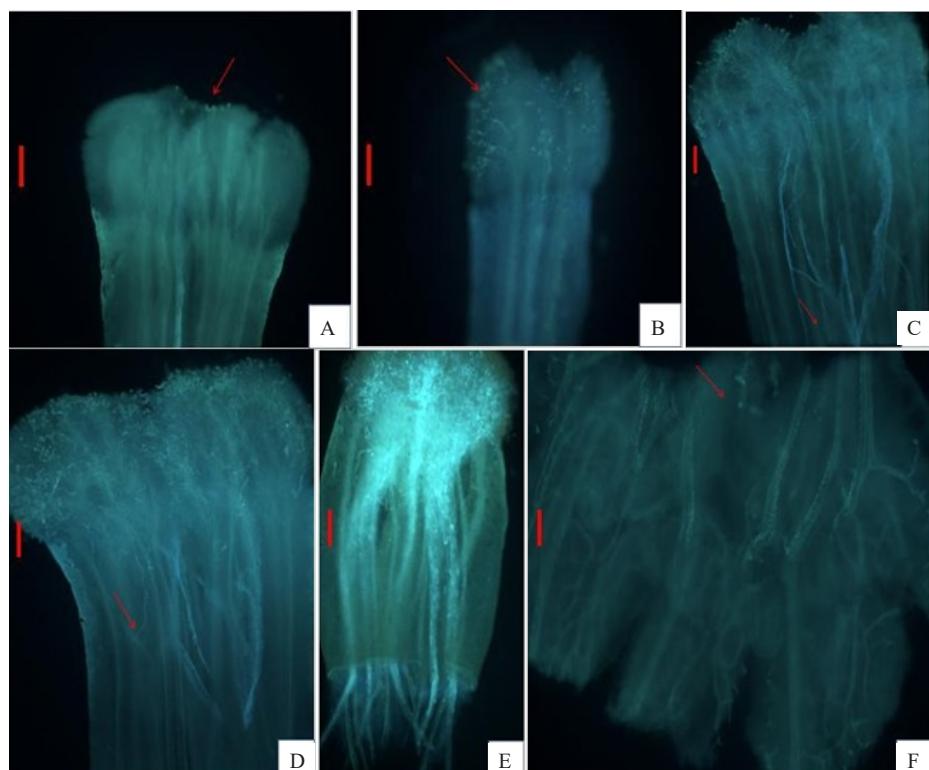
2.4 金柑自交与杂交花粉管生长比较

通过对自交和杂交的花粉管进行观察和比较,发现所有的自交和杂交花粉管在授粉后2 h左右开始萌发(图4-A、B);授粉4~8 h时,杂交花粉管的生长长度达到了花柱总长的1/3~1/2,而自交花粉管生长速度相对较慢;授粉12~24 h时,大多数杂交花粉管已经延伸到了花柱基部(图4-C),一些自交花粉管在花柱总长的2/3处停止了伸长(图4-D)。授粉

48 h后,所有杂交花粉管和部分自交花粉管穿过花柱基部,进入子房内部(图4-E、F),宁波金弹、宁波罗纹CS及牛奶金柑等自交组合中,花粉管生长缓慢甚至停止。上述结果表明金柑种质具有完全杂交亲和性,且存在部分自交不亲和现象。

2.5 不同处理条件下坐果率的比较

在去雄不授粉条件下,7份金柑种质均得到了果实,说明金柑子房在未授粉的情况下也能发育成为果实,具有单性结实的特性;在人工自交授粉处理条件下,蓝山金柑的坐果率最高,并与其他金柑种质



A. 自交 2 h 花粉管生长状;B. 杂交 2 h 花粉管生长状;C. 杂交 24 h 花粉管生长状;D. 自交 24 h 花粉管生长状;E. 杂交 48 h 花粉管生长状;F. 花粉管进入子房。

A. Self-inbred 2 h pollen tube growth state; B. Hybrid 2 h pollen tube growth state; C. Pollen tube growth state in 24 h hybridization; D. Self-inbred 24 h pollen tube growth state; E. Hybrid 48 h pollen tube growth state; F. Pollen tube into the ovary.

图 4 金柑自交和杂交花粉管生长比较

Fig. 4 Comparison of self-crossed and hybrid pollen tube growth of kumquat

间呈现出显著性差异;在杂交授粉处理条件下,浏阳金柑CQ的坐果率最高,其次为蓝山金柑,该2种金柑种质间不存在显著性差异,但与其他5种金柑种质存在显著性差异(图5)。在人工自交授粉、去雄不授粉和杂交授粉三种处理条件下,蓝山金柑得到了较高的坐果率,与蓝山金柑花粉活性和柱头可授性均最高有关。

同一母本材料在人工自交授粉处理及杂交授

粉处理下的坐果率差异性分析如表5,发现除宁波金弹和宁波罗纹CS外,其他5种金柑种质在两种不同处理条件下的坐果率呈现出极显著性差异,且杂交授粉比自交授粉的坐果率更高,说明金柑种质的杂交亲和性比自交亲和性更强,有利于金柑的杂交育种。上述结果与2.4花粉管生长的试验结果相同。

2.6 金柑杂交授粉的坐果率差异性分析

在杂交授粉处理条件下,同一父本不同母本间坐

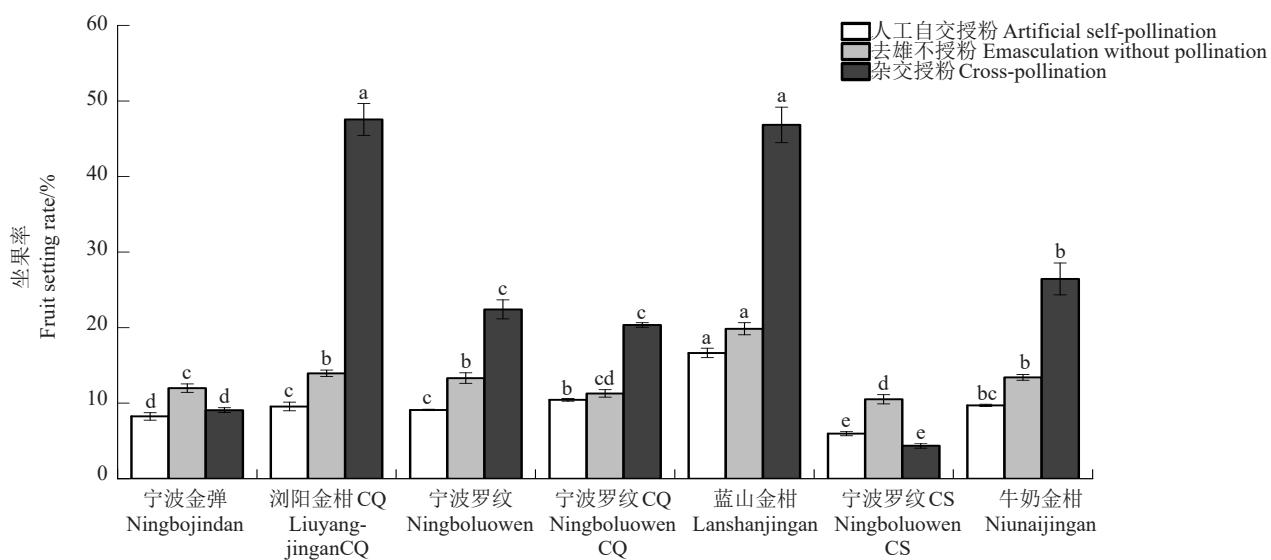


图 5 不同金柑种质的坐果率统计比较

Fig. 5 Statistical comparison of fruit setting rate of different kumquat germplasms

表 5 人工自交授粉与杂交授粉的金柑坐果率差异性分析

Table 5 Differential analysis of fruit setting rate of kumquat between artificial self-pollination and cross-pollination

母本材料 Female parent	不同处理方法的坐果率 Fruit setting rate of different processing method/%		t 值 t-value	p 值 p-value
	人工自交授粉 Artificial self-pollination	杂交授粉 Cross-pollination		
宁波金弹 Ningbojindan	8.25±0.49	9.08±0.31	-2.451	0.070
浏阳金柑 CQ Liuyangjingan CQ	9.56±0.57	47.56±2.11	-30.122	0.000**
宁波罗纹 Ningboluowen	9.10±0.09	22.41±1.26	-18.214	0.000**
宁波罗纹 CQ Ningboluowen CQ	10.44±0.17	20.35±0.30	-49.619	0.000**
蓝山金柑 Lanshanjingan	16.65±0.62	46.84±2.35	-21.900	0.000**
宁波罗纹 CS Ningboluowen CS	5.97±0.29	4.37±0.33	6.37	0.003**
牛奶金柑 Niunaijingan	9.70±0.15	26.44±2.10	-13.79	0.000**

注: **表示极显著($p \leq 0.01$)。下同。

Note: ** indicates highly significant ($p \leq 0.01$). The same below.

果率的差异情况如表 6 所示。父本材料为温州金弹 CS 时,母本材料为宁波金弹与宁波罗纹坐果率呈现极显著性差异,柱头活性高的宁波罗纹坐果率更高;母本材料宁波金弹与宁波罗纹 CQ 坐果率呈现出极显著性差异;宁波罗纹和宁波罗纹 CQ 间坐果率差异并不显著,但宁波罗纹的柱头活性更强,坐果

率更高;父本材料为蓝山金柑时,母本材料为宁波罗纹 CS 与浏阳金柑 CQ 的坐果率存在极显著性差异,尽管宁波罗纹 CS 的柱头活性更高,但坐果率却更低,说明种间杂交亲和性更差;父本材料为浏阳金柑 CQ 时,母本材料牛奶金柑和蓝山金柑的坐果率间存在极显著性差异,蓝山金柑柱头活性更强,且种内杂交亲和性更强,因而其坐果率更高。综上所述,柱头活性越高,坐果率越高,且种内杂交的坐果率高于种间杂交。因此在选择杂交亲本时,应考虑柱头活性和亲本亲缘关系等因素。

3 讨 论

了解植物授粉生物学特性对植物杂交育种具有重要的指导意义^[20, 25-27]。对于柑橘类植物的杂交育种来说,经常由于珠心胚的干扰而不能获得杂种,其原因是珠心胚与合子胚之间存在竞争关系,导致合子胚发育失败^[28]。但当以单胚为母本时可以提高柑橘类植物的杂种率,因此笔者在本研究中比较了不同金柑种质的胚数,以筛选出单胚种质,作为杂交的母本材料。笔者在本研究中发现罗纹种为单胚种质,适合作为杂交母本。

柱头可授性和花粉活性也是影响杂交成功率的重要因素。通过比较不同时期金柑花蕾的柱头可授性和花粉活性,结果显示二者的活性趋势相似,且均在初开期达到最强,属于雌雄同熟植物,并未发现花粉败育情况^[29]。试验结果与百香果和樱桃相

表6 金柑不同杂交组合间坐果率差异性分析

Table 6 Variability analysis of fruit setting rate among different hybrid combinations of kumquat

父本材料 Male parent	母本材料 Female parent	柱头可授性得分 Stigma receptivity score	坐果率(平均值±标准差) Fruit setting rate (mean ± standard deviation)/%	t值 t-value	p值 p-value
温州金弹 CS Wenzhoujindan CS	宁波金弹 Ningbojindan	5.00	9.08±0.31	-17.763	0.000**
	宁波罗纹 Ningboluowen	5.67	22.41±1.26		
	宁波金弹 Ningbojindan	5.00	9.08±0.31	-45.108	0.000**
	宁波罗纹 CQ Ningboluowen CQ	5.00	20.35±0.30		
	宁波罗纹 CQ Ningboluowen CQ	5.00	20.35±0.30	-2.749	0.051
蓝山金柑 Lanshanjingan	宁波罗纹 CS Ningboluowen CS	5.33	4.37±0.33	-35.039	0.000**
	浏阳金柑 CQ Liuyangjingan CQ	4.67	47.56±2.11		
	牛奶金柑 Niunaijingan	5.67	26.44±2.10	-11.209	0.000**
浏阳金柑 CQ Liuyangjingan CQ	蓝山金柑 Lanshanjingan	6.00	46.84±2.35		

同^[30-31]。但是在杂交实践中,为了避免串粉,通常在初开期之前的大蕾期进行花粉采集和去雄处理。

被子植物受精过程主要包括花粉与柱头的识别、花粉管在花柱间的伸长,以及雌雄配子间的融合三个阶段,任意阶段无法识别均会导致杂交失败^[32]。在授粉后2 h内为花粉与柱头识别的重要时间段^[33],笔者在本研究中发现,金柑在授粉处理2 h后,所有自交和杂交均完成了花粉与柱头的识别,花粉管开始在花柱内生长。在授粉48 h后,所有杂交花粉管和部分自交花粉管穿过花柱基部进入子房。在花粉管的行为上表现出了较好的杂交亲和性,该现象在多种植物中被发现^[34-35]。在本研究中,自交花粉管在花柱内停滞生长,表现出自交不亲和的现象,同样被雷翠云^[36]发现。在花粉管生长阶段导致果树自交不亲和的因素有很多^[37],目前对金柑中花粉管生长停滞现象的原因尚未见报道。在柑橘类植物中,被证实S-RNase基因的表达调控^[38]和信号传导^[39]均会对花粉管的发育产生影响。其他原因如花粉管的导向生长^[40]、微丝、微管骨架^[41]等也会导致花粉管的生长停滞。

坐果率能直接反映出种质间亲和性的强弱,是研究种质间杂交及自交亲和性的重要指标之一^[42-44]。笔者在本研究中发现,大部分金柑杂交的坐果率高于自交,并呈现极显著差异,该现象的原因可能是金柑具有杂交亲和性以及部分自交不亲和性,并且与花粉管的生长观察试验结果相同。该现象已在植物中被发现,如梨树^[45]、甜菊^[46]、枣^[47]等。并且笔者在本研究中还发现,对金柑进行去雄不授粉处理后,子房仍可以发育成果实,表明金柑具有单性结实

的特性,与雷翠云^[36]的研究结果相同。

植物柱头的可授性强弱可以直接影响坐果率的高低,也是决定杂交育种工作成功与否的重要指标之一^[48-49]。笔者在本研究中发现柱头活性越强,坐果率越高,且种内杂交的坐果率高于种间杂交。相关研究也表明,柱头可授性与坐果率间存在显著正相关^[26, 50]。因此在选择杂交亲本时,应考虑柱头活性和亲本亲缘关系等因素。以上对金柑杂交的生物学特性研究,为金柑杂交条件的确定以及杂交亲本的选择提供了一定的参考依据。

4 结 论

金柑杂交中应选择单胚型母本材料;金柑属于雌雄同熟果树,采集花粉最佳时期和授粉最佳时期均为初开期;金柑杂交亲和性比自交亲和性更强,有利于金柑的杂交育种。

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