果 树 学 报 2023,40(9):1832-1838
Journal of Fruit Science

DOI:10.13925/j.cnki.gsxb.20230143

荔枝雄性不育株系MS1的发现和鉴定

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摘 要:【目的】对新发现的荔枝雄性不育株系MS1进行鉴定,为后续研究利用奠定基础。**【方法】**以荔枝雄性不育株系MS1为研究材料,以雄性可育株系D13为对照,通过外观比较、扫描电镜观察、研磨花药镜检、花药石蜡切片等方法对MS1株系雄性不育进行鉴定。**【结果】**荔枝雄性不育株系MS1雄花数量少、个头小、花药瘦小、表面多凹陷、成熟后多不开裂、药室内无花粉粒且自交无坐果。**【结论】**荔枝雄性不育株系MS1为无花粉型雄性不育。

关键词:荔枝;雄性不育;花药;花粉

中图分类号:S667.1 **文献标志码:**A **文章编号:**1009-9980(2023)09-1832-07

Discovery and identification of litchi male sterile line MS1

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Abstract:【Objective】Male sterile line plays an important role in cross breeding and heterosis utilization of plants. In recent years, we discovered a new litchi male sterile line and named it MS1. In this study, we identified its sterile characters by various methods, so as to provide the foundation for the following research and use.【Methods】Using the grafted litchi male sterile line MS1 and male fertile line D13 as experimental materials. Using Sony α77 camera and self-made macro lens to take photos of flower organs, setting the same shooting distance and exposure to ensure the actual size and color were reflected. The different appearances of litchi male sterile line MS1 and the male fertile line D13 were analyzed. Using 1% safranine solution with 75% alcohol for anther staining. After staining, using 10% sucrose solution with distilled water to reduce the surface tension. Then the anthers were ground and observed under a microscope. The paraffin section was carried out for observation of the anther structure. The samples was dehydrated with 70%, 80%, 95% and 100% alcohol for 15 minutes each, then transparentized with xylene for 15 minutes twice, and an 8 μm permanent section was made. The microscope was used to magnify 100−200 times for observation and photography. The silicone dryer was used to dry the anthers for 72 hours, after coating and spraying gold, the samples were observed under the SEM. 1% sucrose solution was used to dissolve the prepared anther powder of D13 and MS1, MS1 female spikes were sprayed with the two solutions respectively and bagged up. The number of fruit setting was counted 30 days after the pollination.【Results】(1) In appearance, litchi male sterile line MS1 had a small number of male flowers, anthers, and its anther color was milky white, which could easily fall off without cracking. The number of anthers of MS1 was 4−6, while the number of anthers of D13 was 6−12. Occasionally some spikes of MS1 only come out with female flowers. A few anthers might

收稿日期:2023-04-14 **接受日期:**2023-05-11

基金项目:海南省自然科学基金(323QN294,320QN321);国家现代农业(荔枝龙眼)产业技术体系(CARS-32-17)

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crack when mature, but no pollen grains could be observed. The litchi fertile line D13 had a large number of male flowers and its anther color was faint yellow, which would easily cracke when mature and release a large amount of pollen; (2) Under the SEM, the female and male anthers of the litchi male sterile line MS1 were thin and small, with many sunken surfaces, and most of them did not crack when mature. The anther chambers of the litchi male fertile line D13 cracked when mature where there were a large number of pollen grains; (3) After aniline safranine staining, no pollen grains were found in the anther grinding fluid of the litchi male sterile line MS1, while a large number of different color of pollen grains were found in the anther grinding fluid of the litchi male fertile line D13; (4) Paraffin sections showed that there was no pollen grain in the anther chamber of the litchi male sterile line MS1, with normal epidermis, endothecium and vascular bundle, and the anther chamber formed a cavity and did not crack when mature. The litchi fertile line D13 had normal epidermis, endothecium, and vascular bundle. The Pollen grains were released from the cracking stomium of the anther chamber when mature; (5) The litchi male sterile line MS1 had a failure in fruiting with self-pollination, while litchi male sterile line MS1 had normal fruit setting (8.2 fruits of each spike averagely) when it was pollinated with the pollens of the litchi male fertile line D13.【Conclusion】Compared with the normal fertile line, litchi male sterile line MS1 was a male sterile of pollen-free with normal epidermis, endothecium and vascular bundle, and its anther chamber formed a cavity and did not crack when mature.

Key words: Litchi; Male sterile; Anther; Pollen

荔枝是起源于我国云南的常绿果树，已有2000多年的种植记载历史[1]。近30年来，国内育成荔枝新品种38个，这些品种多为从资源普查期间的实生系和营养系的扩繁而来[2]。但该选种方法周期长、效率低，目前已进入瓶颈期，这也促进了各地陆续开展规模化人工杂交育种工作[3]，杂种优势的利用逐渐得到人们的重视。

雄性不育种质在杂种优势利用中有重要地位。荔枝是雌雄同株的异花授粉作物，有明显的“雄花-雌花-雄花”分批开放的现象[4]。在杂交育种中，常用乙烯利减少雄花配合人工剔除的方法进行荔枝去雄[5]，剂量不仅难以精准控制，而且费时费力，工作效率不高。利用雄性不育材料进行杂交育种，不仅能免去繁杂的去雄操作，得到真杂种的概率也更高。但是，荔枝雄性不育种质目前未见报道。笔者前期在育种材料中发现了一个花药彻底败育（无花粉）的荔枝雄性不育株系，暂命名为MS1。荔枝雄性不育株系MS1易成花、特早熟、单性结实能力强，有弥补市面上早熟、无核荔枝果品空白的潜力。笔者在本研究中参照前人在其他作物上使用的方法[6-10]，对荔枝雄性不育株系MS1进行鉴定，为研究荔枝雄性不育材料奠定良好开端，为后续研究利用打下基础。

1　材料和方法

1.1　植物材料

试验所用材料来源于中国热带农业科学院南亚热带作物研究所荔枝种植资源圃。荔枝雄性不育株系MS1树龄5~10 a，生长良好。剪取MS1接穗，嫁接到广东广州、阳江的试验基地进行区域试验。选取与荔枝雄性不育株系MS1物候期相近的荔枝雄性可育株系D13作为对照，树龄5~10 a，生长良好。

1.2　显微镜检方法

摘取D13和MS1株系新鲜荔枝花穗，带回实验室后，选取雌花柱头开裂、雄花花朵开放（花蜜分泌）阶段的花器官，用镊子将花药剥离，加入适量无菌水和苯胺番红溶液捣碎，在显微镜下观察。

1.3　扫描电镜观察方法

摘取D13和MS1株系新鲜荔枝花穗，带回实验室后，选取雌花柱头开裂、雄花花朵开放（花蜜分泌）阶段的花器官，将花药剥离，置入硅胶干燥器干燥72 h后，将其转移至贴有导电胶的铜台上，真空镀膜喷金，用扫描电镜拍照。

1.4　石蜡切片方法

新鲜荔枝花器官摘下后投入甲醛-乙酸-乙醇

（formaldehyde-acetic acid-ethanol，FAA）固定液中固定24 h以上，经脱水、透明、浸蜡、包埋、切片、展片、脱蜡、染色和封片等常规制片程序处理后，制成荔枝花器官永久切片，用于观察拍照。

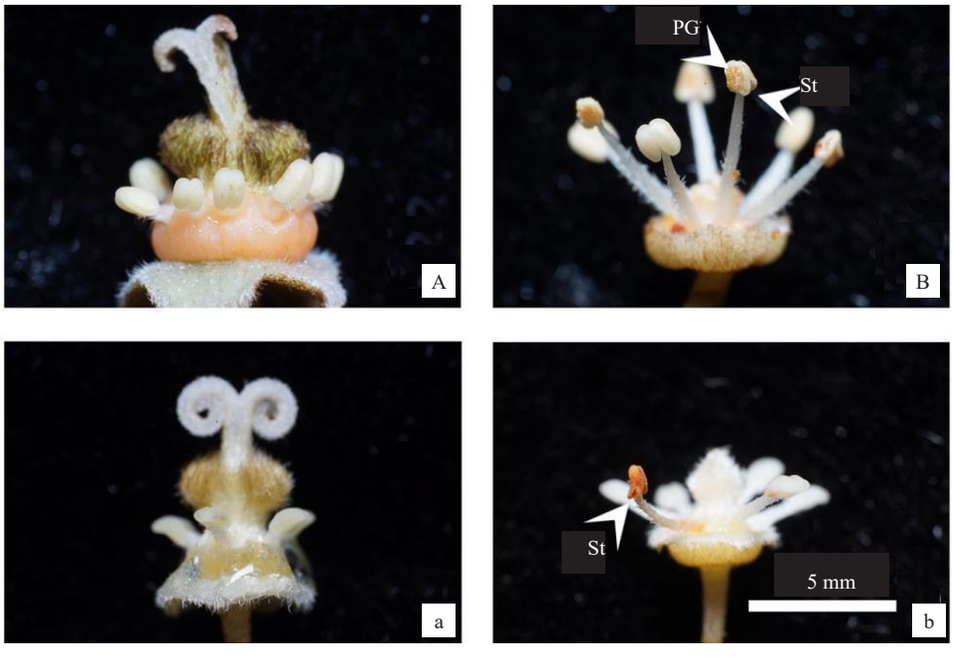
1.5 杂交授粉方法

摘取新鲜荔枝花穗，带回实验室后，选取雄花花丝伸长至最长、花朵开放（花蜜分泌）阶段的花器官，将花药剥离，尽量去除花丝、花柄等杂质，30 ℃鼓风干燥12 h后，过0.075 mm筛网，收集下层物质溶于1%（*w*，后同）蔗糖溶液，再经0.075 mm筛网过滤后，喷施于柱头刚裂开的母本花穗并套袋。

2 结果与分析

2.1 荔枝雄性可育株系D13和雄性不育株系MS1花器官外观特征

对荔枝雄性可育株系D13和雄性不育株系MS1雌、雄花器官进行观察比较。荔枝雄性可育株系D13雌、雄花药呈淡黄色，雄花器官大、数量多、花丝长且花药数量多（6~12个），花朵开放（花蜜分泌）后雄花花药逐渐褐变、开裂，裂口处可见大量金黄色花粉粒（图1-A~B）；荔枝雄性不育株系MS1雌、雄花药呈乳白色，雄花器官小、数量少、花丝短且花药数量少（4~6个）（图1-a~b），该特征符合植物雄性不育一般特征。值得注意的是，荔枝雄性不育株系MS1绝大多数雄花花药发育至花朵开放阶段（花蜜分泌）仍为乳白色，不开裂，此时雄花即脱落，极少数提前发育的花药可褐变并开裂，但裂口处未见花粉粒。



A. 荔枝雄性可育株系 D13 雌花；B. 荔枝雄性可育株系 D13 雄花；a. 荔枝雄性不育株系 MS1 雌花；b. 荔枝雄性不育株系 MS1 雄花。PG. 花粉粒；St. 裂口。

A. Female flower of litchi male fertile line D13; B. Male flower of litchi male fertile line D13; a. Female flower of litchi male sterile line MS1; b. Male flower of litchi male sterile line MS1. PG. Pollen grains; St. Stomium.

图1 荔枝雄性可育株系D13和雄性不育株系MS1雌、雄花器官

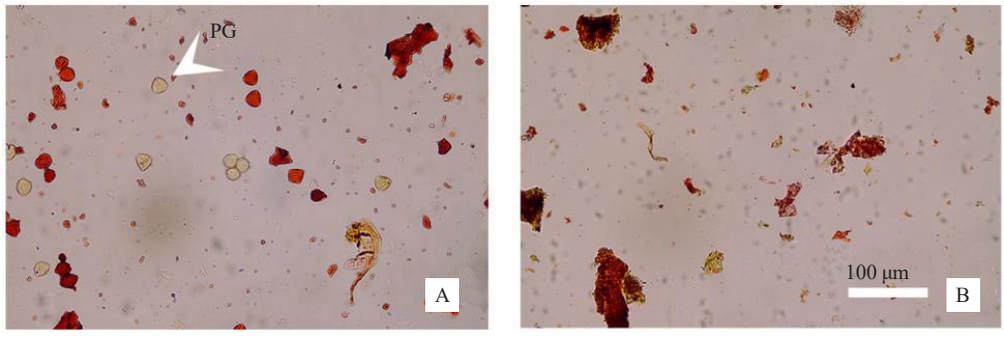
Fig. 1 Female and male flower of male fertile line D13 and male sterile line MS1 in litchi

2.2 荔枝雄性可育株系D13和雄性不育株系MS1花药研磨液显微镜观察

对荔枝雄性可育株系D13和雄性不育株系MS1花药进行染色、研磨。荔枝雄性可育株系D13花药研磨液中可见大量颜色深浅不一的花粉粒和组织碎片（图2-A）；荔枝雄性不育株系MS1花药研磨液中未见花粉粒，仅可见组织碎片（图2-B）。

2.3 荔枝雄性可育株系D13和雄性不育株系MS1花药扫描电镜观察

对荔枝雄性可育株系D13和雄性不育株系MS1雌、雄花药进行扫描电镜观察。荔枝雄性可育株系D13雄花花药饱满、表面纹理平整光滑，花药成熟后药室现裂口，可见大量花粉粒（图3-A）；荔枝雄性可育株系D13雌花花药饱满、表面纹理平整光滑，成熟

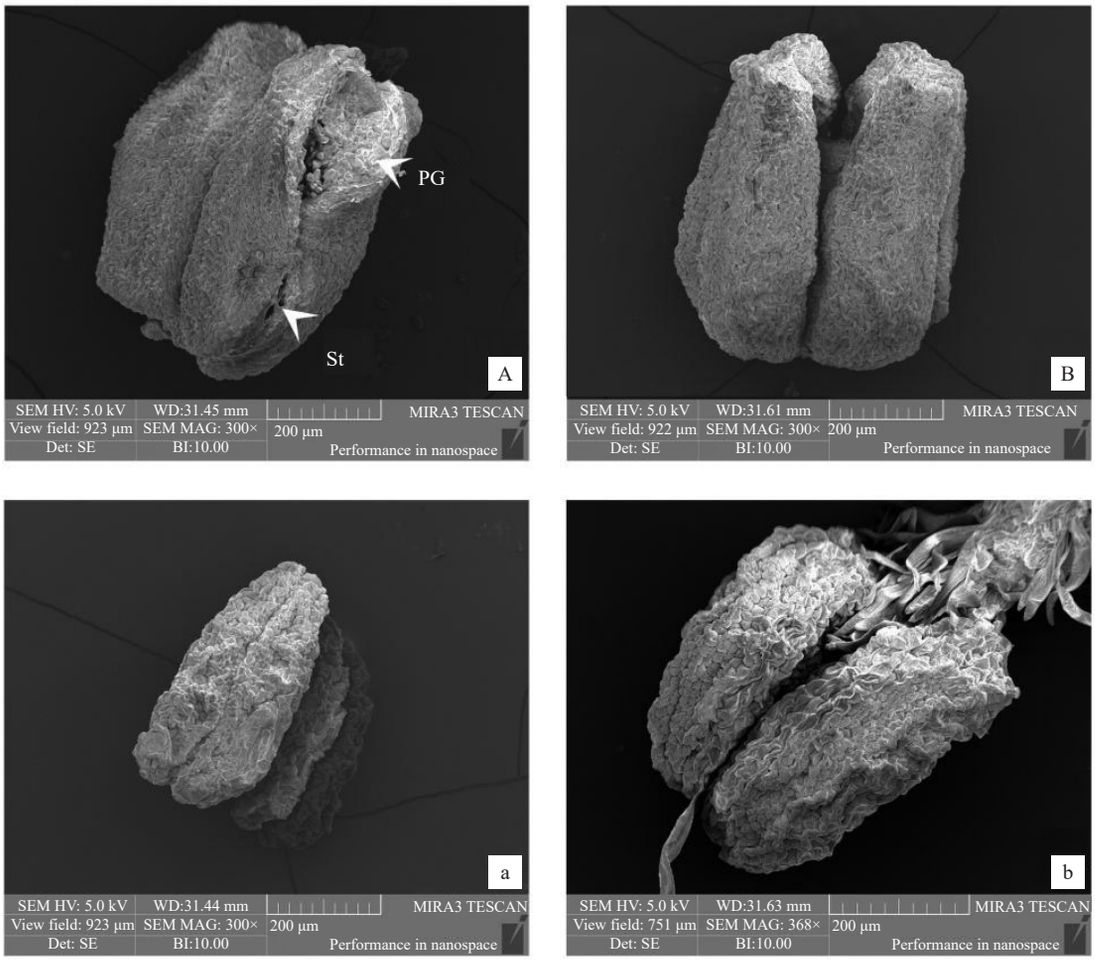


A. 荔枝雄性可育株系 D13 花药研磨液；B. 荔枝雄性不育株系 MS1 花药研磨液。PG. 花粉粒 .

A. Anther grinding liquid of litchi male fertile line D13; B. Anther grinding liquid of litchi male sterile line MS1. PG. Pollen grains.

图 2　荔枝雄性可育株系 D13 和雄性不育株系 MS1 花药研磨液显微镜检

Fig. 2　Microscopic examination of anther grinding liquid of male fertile line D13 and male sterile line MS1 in litchi



A. 荔枝雄性可育株系 D13 雄花花药；B. 荔枝雄性可育株系 D13 雌花花药；a. 荔枝雄性不育株系 MS1 雄花花药；b. 荔枝雄性不育株系 MS1 雌花花药。BI. 电子束强度；Det. 直接电子转移过程；HV. 加速电压；MAG. 放大倍数；PG. 花粉粒；SE. 二次电子探测器；SEM. 扫描电镜；St. 裂口；WD. 工作距离。

A. Anther of male flower of litchi male fertile line D13; B. Anther of female flower of litchi male fertile line D13; a. Anther of male flower of litchi male sterile line MS1; b. Anther of female flower of litchi male sterile line MS1. BI. beam intensity; Det. Direct electron transfer process; HV. High voltage; MAG. Magnification; PG. Pollen grains; SE. Secondary electron detector; SEM. Scanning electron microscope; St. Stomium; WD. Work distance.

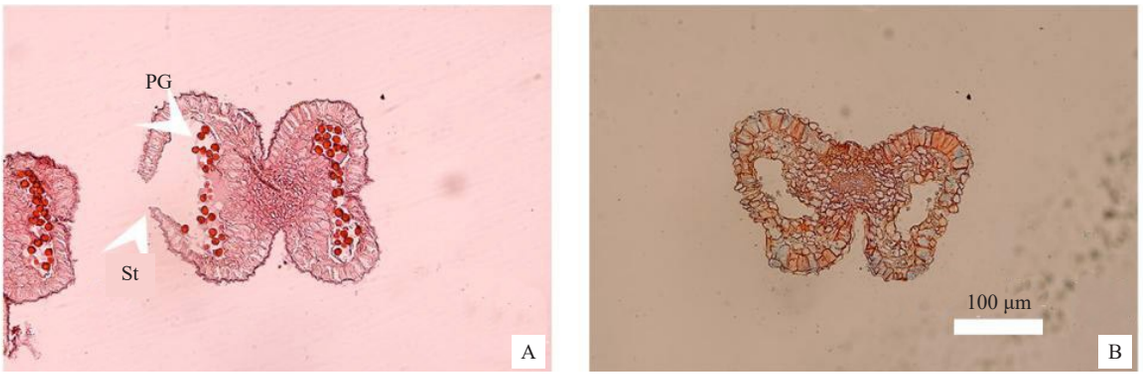
图 3　荔枝雄性可育株系 D13 和雄性不育株系 MS1 花药扫描电镜观察

Fig. 3　Litchi anther SEM observation of male fertile line D13 and male sterile line MS1

后花药不开裂（图3-B）；荔枝雄性不育株系MS1雄花花药瘦小、表面纹理不光滑且凹陷多，成熟后花药不开裂（图3-a）；荔枝雄性不育株系MS1雌花花药瘦小、表面纹理不光滑、凹陷多，花药与花丝不易脱离，成熟后花药不开裂（图3-b）。

2.4 荔枝雄性可育株系D13和雄性不育株系MS1花药石蜡切片观察

对荔枝雄性可育株系D13和雄性不育株系MS1成熟雄花花药（花丝伸长到花朵开放阶段）进行石蜡切片，观察结构差异。荔枝雄性可育株系D13雄花花药成熟后，药室逐渐开裂，药室内大量花粉粒从裂口处释放（图4-A）；荔枝雄性可育株系D13雄花花药成熟后，药室形成空腔，未见花粉粒，花药不开裂（图4-B）。



A. 荔枝雄性可育株系 D13 雄花花药结构；B. 荔枝雄性不育株系 MS1 雄花花药结构。PG. 花粉粒；St. 裂口。

A. Anther structure of male flower of litchi male fertile line D13; B. Anther structure of male flower of litchi male sterile line MS1. PG. Pollen grains; St. Stomium.

图4 荔枝雄性可育株系D13和雄性不育株系MS1花药结构

Fig. 4 Structure of anther of male fertile line D13 and male sterile line MS1 in litchi

2.5 雄性不育株系MS1作为母本搭配不同父本坐果情况

按照常规采粉和授粉的方法，采集荔枝雄性可育株系D13和雄性不育株系MS1成熟花药，烘干后过筛，溶于1%蔗糖溶液后向荔枝雄性不育株系MS1雌花盛开阶段的花穗喷施后套袋，30 d后进行比较。喷施自身雄花花药溶液进行自交的MS1花穗，无坐果（图5-A）；喷施D13花粉溶液进行杂交的

A. 喷施自身雄花花药溶液 30 d 后的雄性不育株系 MS1 穗部；B. 喷施雄性可育株系 D13 雄花花粉溶液 30 d 后的雄性不育株系 MS1 穗部。

A. Spike of male sterile line MS1 sprayed with its own male anther solution for 30 d; B. Spike of male sterile line MS1 sprayed with male fertile line D13 male anther solution for 30 d.

图5 喷施不同花药研磨液后荔枝雄性不育株系MS1坐果情况

Fig. 5 After spraying different anther grinding fluids fruit setting of litchi male sterile line MS1

MS1花穗，坐有大量小果（图5-B）。

3　讨　论

雄性不育资源在杂种优势利用中有重要地位。新的雄性不育资源的发现，为研究和理解遗传进化提供了重要材料[11]。一直以来，前人在荔枝雌花败育[12]、乙烯诱导雄花败育[13]、花性分化[13-15]等多个方面均进行了相似的研究，但鉴于理想的材料一直未见报道，许多研究无法深入推进。荔枝雄性不育株系MS1，是荔枝花发育领域研究和育种工作的重要材料。笔者以该株系为母本，培育了一批实生子一代群体，根据以往经验，其子一代应具有相当比例的育性保持或分离的植株，这为后续关键基因克隆及新的荔枝雄性不育材料的创制、研究和利用打下了必要的理论和实践基础。

值得一提的是，笔者发现的荔枝雄性不育株系MS1是较为理想的花药败育材料，其不仅彻底败育（无花粉），还以多种方式体现了雄花失能。一是雄花数量少；二是雄花花药数量少（4~6个），雄性可育株系雄花花药数量为6~12个；三是MS1雄花花药发育至末期不变色、开裂，花柄易脱落，而对照雄性可育株系D13雄花花药在发育至末期变色、开裂，花柄不易脱落，持续散粉1~2 d。笔者还发现该株系在广东广州、阳江均表现为无花粉型雄性不育，说明其不育性状稳定，在粤中、粤西地区自然气候条件下育性不受影响，但其育性是否可通过温光调控进行转换有待进一步确认。

除在基础研究方面的重要意义外，荔枝雄性不育株系MS1还存在一定应用价值，其成花需冷量低、特早花且有单性结实能力，花期只需套袋配合保果措施即可得到完全无核的荔枝果实，有填补市面上早熟、无核荔枝果品空白的潜力，但由于单性结实无核荔枝果实未经受精授粉，果实易脱落，配套的生产栽培技术尚需进一步研发。

4　结　论

荔枝雄性不育株系MS1在广东湛江本地表现无花粉型雄性不育，其雄花数量少、个头小、花药瘦小、表面多凹陷、成熟后多不开裂且药室内无花粉粒，符合植物雄性不育的一般特征，不育性状在本地自然栽培条件下多年表现稳定，是较为理想的研究利用材料。

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