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3个猕猴桃栽培种花粉形态扫描电镜观察

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摘 要:【目的】明确3个猕猴桃栽培种的花粉形态学特征,了解其遗传变异特性、亲缘关系,为花粉的外观形态与种间分类提供依据。【方法】利用扫描电镜对30个品系的花粉粒进行形态观察。【结果】花粉粒外观为长球形或超长球形,极面观均为三裂圆形,可看到3条内陷萌发沟。美味猕猴桃萌发沟长度18.08~31.48 μm、萌发沟脊宽度4.65~11.42 μm、极轴长度21.54~36.05 μm、赤道轴长度9.60~17.66 μm,极轴/赤道轴1.65~3.05、大小244.90~608.52 μm²。中华猕猴桃萌发沟长度14.09~24.96 μm、萌发沟脊宽度4.09~9.37 μm、极轴长度15.60~30.11 μm、赤道轴长度8.85~17.87 μm、极轴/赤道轴1.26~2.65、大小182.58~443.18 μm²。软枣猕猴桃萌发沟长度16.34~30.06 μm、萌发沟脊宽度6.11~11.23 μm、极轴长度18.66~32.11 μm、赤道轴长度8.76~18.42 μm、极轴/赤道轴1.33~2.68、大小166.62~547.07 μm²。表面纹饰存在3种类型:(1)波纹状,美味猕猴桃、中华猕猴桃和软枣猕猴桃样品均包含,占样品总数的53.33%;(2)疣状,美味猕猴桃、中华猕猴桃和软枣猕猴桃样品均包含,占样品总数的36.67%;(3)颗粒状,均为软枣猕猴桃,占样品总数的10.00%。【结论】猕猴桃花粉外观形态和大小在相同种内和相同来源环境背景下存在一定的保守性;整体来看,美味猕猴桃花粉最大,其次是软枣猕猴桃花粉,显著大于中华猕猴桃花粉;花粉大小、萌发沟长度、萌发沟脊宽度、极轴长度、赤道轴长度,中华猕猴桃均显著小于美味猕猴桃,可作为2者划分的孢粉学特征依据。

关键词:猕猴桃;花粉形态;纹饰;遗传多样性

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Morphologic study of pollens of three cultivated *Actinidia* species by scanning electron microscopy

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Abstract:【Objective】In order to obtain the knowledge of genetic variation and relationship of various cultivars in the genus *Actinidia* Lindl., the pollen morphological characteristics of three *Actinidia* species were investigated.【Methods】Scanning electron microscopy was used to observe pollen morphology of thirty samples collected from three cultivated *Actinidia* species, *A. deliciosa*, *A. chinensis* and *A. arguta*. Parameters of pollen grains such as the length of polar axis, length of equatorial axis, length of colpus and width of colpus were measured and analyzed using an image software (Imagepro-Plus).【Results】The pollens of *A. deliciosa*, *A. chinensis* and *A. arguta* were in the form of a single grain, being prolate or perprolate. Only one sample of *A. deliciosa* contained the prolate-shaped pollen grains whereas all samples of *A. chinensis* contained the perprolate-shaped pollen grains. A total of ten samples of *A. arguta* were included in this study, four of them contained the prolate-shaped pollen grains while others contained perprolate-shaped pollen grains. Each pollen grain contained three colpi which elongated along the polar axis till

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they reached the ends. One or two colpi could be observed in the equatorial view, whereas all three corpi that were furrows in trifid circular could be seen in the polar view. The results of the measurements of *A. deliciosa* pollen grain were as follows: The length of colpus was 18.08-31.48 μm and the coefficient variation was 9.59%. The width of colpus was 4.65-11.42 μm and the coefficient variation was 12.40%. The length of polar axis was 21.54-36.05 μm and the coefficient variation was 7.6%. The length of equatorial axis was 9.60-17.66 μm and the coefficient variation was 10.01%. The ratio between the polar and the equatorial axis was 1.65-3.05 and the coefficient variation was 9.68%. The pollen size was 244.90-608.52 μm^2 and the coefficient variation was 15.06%. The results of the measurements of *A. chinensis* pollen grain were as follows: The length of colpus was 14.09-24.96 μm and the coefficient variation was 14.00%. The width of colpus was 4.09-9.37 μm and the coefficient variation was 15.88%. The length of polar axis was 15.60-30.11 μm and the coefficient variation was 11.17%. The length of equatorial axis was 8.85-17.87 μm and the coefficient variation was 11.80%. The ratio between the polar and the equatorial axis was 1.26-2.65 and the coefficient variation was 9.93%. The pollen size was 182.58-443.18 μm^2 and the coefficient variation was 21.52%. The results of the measurements of *A. arguta* pollen grain were as follows: the length of colpus was 16.34-30.06 μm and the coefficient variation was 11.62%. The width of colpus was 6.11-11.23 μm and the coefficient variation was 14.13%. The length of polar axis was 18.66-32.11 μm and the coefficient variation was 9.21%. The length of equatorial axis was 8.76-18.42 μm and the coefficient variation was 10.79%. The ratio between the polar and the equatorial axis was 1.33-2.68 and the coefficient variation was 10.90%. The pollen size was 166.62-547.07 μm^2 and the coefficient variation was 16.70%. There are three types of surface ornamentation in *Actinidia* pollens. (1) The corrugated ornamentation was covered with a smooth surface composed of irregular strips. Sixteen samples showed corrugated ornamentation, which accounted for 53.33% of the total samples used in this study. Among them, five samples belonged to *A. deliciosa*, which accounted for 38.46% of *A. deliciosa* pollens. Five samples belonged to *A. chinensis*, which accounted for 71.43% of *A. chinensis* pollens. Seven samples belonged to *A. arguta,* which accounted for 70% of *A. arguta* pollens. (2) The pollen with warty ornamentation was covered by regular or irregular block like protrusions. The size of these protrusions was irregular. Eleven samples showed warty ornamentation, which accounted for 36.67% of all samples included in this study. Eight samples belonged to *A. deliciosa*, which accounted for 61.54% of *A. deliciosa* pollens. Two samples belonged to *A. chinensis, which* accounted for 10% of *A. chinensis* pollens. One sample belonged to *A. arguta*, which accounted for 10% of *A. arguta* pollens. (3) The surface of pollens with granular ornamentation was distributed with particles or in the pattern of granules. Three samples showed granular ornamentation, which accounted for 10% of all samples included in this study. The samples with granular ornamentation belonged to *A. arguta* which was originated from the province of Heilongjiang. It accounted for 30% of *A. arguta* pollens.【Conclusion】There was a certain degree of conservation in pollen morphology and size of *Actinidia* pollens derived from the same species or environmental background. Overall, the pollen size of *A. deliciosa* was the largest among these species, followed by *A. arguta and A. chinensis*. The length the polar axis, length of equatorial axis, length of colpus and width of colpus, of *A. chinensis* were lower than those of *A. deliciosa*. Hence, the morphological differences of pollens between *A. chinensis* and *A. deliciosa* would provide some evidence for taxonomy of these species.

Key words: Kiwifruit; Pollen morphology; Pollen ornamentation; Genetic diversity

花粉是植物繁殖器官的一部分，其结构由物种的基因所决定，不受或受环境影响很小[1]。花粉大小、形状、对称性和极性，花粉萌发孔的数量、结构和位置，花粉壁的结构以及表面雕纹等，可为植物系统发育的研究提供科学依据[2]。早在1989年，李洁维等[3]用光学显微镜对猕猴桃属(*Actinidia* Lindl.)植物23个分类群的花粉形态进行了观察，但是由于仪器设备的局限性，未对花粉的表面纹饰做详细的表述。之后，相关学者利用扫描电镜观察了猕猴桃不同种花粉的形态特征，并发现其外壁纹饰存在差异，认为可作为属以下分组分类的重要依据，同时根据花粉形态特征建立了相应的检索表[4-7]。分析上述结果，均是基于种间单株取样进行花粉形态学的比较研究和种间分类。但是，对于猕猴桃属植物而言，即使相同种内某些性状方面也存在较大差异，‘红阳’猕猴桃及其芽变单株‘86-3’在花粉的极轴长与赤道轴长之比上存在显著差异[8]；同一自然保护区内的23份野生毛花猕猴桃雄株花粉尽管形态有较多共性特征，但不同单株的花粉外壁纹饰间存在差异，体现了毛花猕猴桃的遗传保守性和多样性[9]。笔者在前期研究中发现，中华猕猴桃同一品种‘琼浆’种子航天搭载后，其实生后代雄株花粉粒极轴长度变异系数为11.65%[10]。为进一步明确猕猴桃花粉的外观形态与种间分类的关系，笔者以生产中最常见的3个栽培种的30份花粉为试材，对上述结论作进一步的验证和补充，为该属分类学研究提供新的科学依据。

1　材料和方法

1.1　试材及取样

试材全部来源于国家农作物种质资源平台——郑州猕猴桃子平台(113°71′E，34°71′N)。选用花粉属于3个猕猴桃种：美味猕猴桃(*A. deliciosa*)、中华猕猴桃(*A. chinensis*)和软枣猕猴桃(*A. arguta*)，各来源雄性单株具体见表1。各单株2009年进入始花期，试验于2016年春季进行。该圃采用水平大棚架管理，园区土壤为砂壤土，中性偏碱，所有树体管理水平一致。

1.2　方法

1.2.1　*花粉采集*　在4—5月雄株的初花期，采摘即将开放的花蕾，将花药用小镊子剥至有盖培养皿中，在25 ℃通风孵化器内放置24 h，轻轻摇晃培养皿，待花粉完全散出后，放置在小玻璃瓶内-20 ℃下密闭保存[10]。

1.2.2　*扫描电镜的观察*　试验在河南农业大学国家小麦工程中心进行。先将花粉样品在2.5%的戊二醛水溶液中浸泡2 h，然后用pH 7.2的PBS缓冲溶液清洗，再用30%、50%、75%、90%和100%的乙醇梯度脱水，每个浓度浸泡15 min，3次重复，然后冷冻干燥。将干燥的花粉粒样品用棉签直接黏在贴有导电双面胶的载台上，在日立S-3400扫描电子显微镜进行下观察，选取典型花粉粒进行拍照。500×视野下对花粉粒整体进行拍照，1 000×视野下对少量花粉进行拍照，4 000×视野下对单个花粉粒进行拍照，10 000×视野下对花粉纹饰进行拍照。

1.2.3　*数据分析*　1 000×视野中，用分析测量图像软件Imagepro-Plus进行花粉粒极轴长(P)、赤道轴长(E)、萌发沟长度、萌发沟脊宽度测量，每种花粉随机测量30粒。试验所得数据采用SPSS 17.0统计软件进行分析，采用Duncan新复极差检验法进行显著性检验。花粉形状的分类方法及学术用语参照《孢粉学概论》[11]：花粉极轴长度10~25 μm属于小，25~50 μm属于中等；极轴长和赤道轴长之比(P/E)表示花粉粒形状(P/E>2表示花粉粒为超长球形，P/E为1.14~2表示花粉粒为长球形)。花粉粒大小用极轴长和赤道轴长(P×E)表示。以最小值至最大值表示变异幅度。

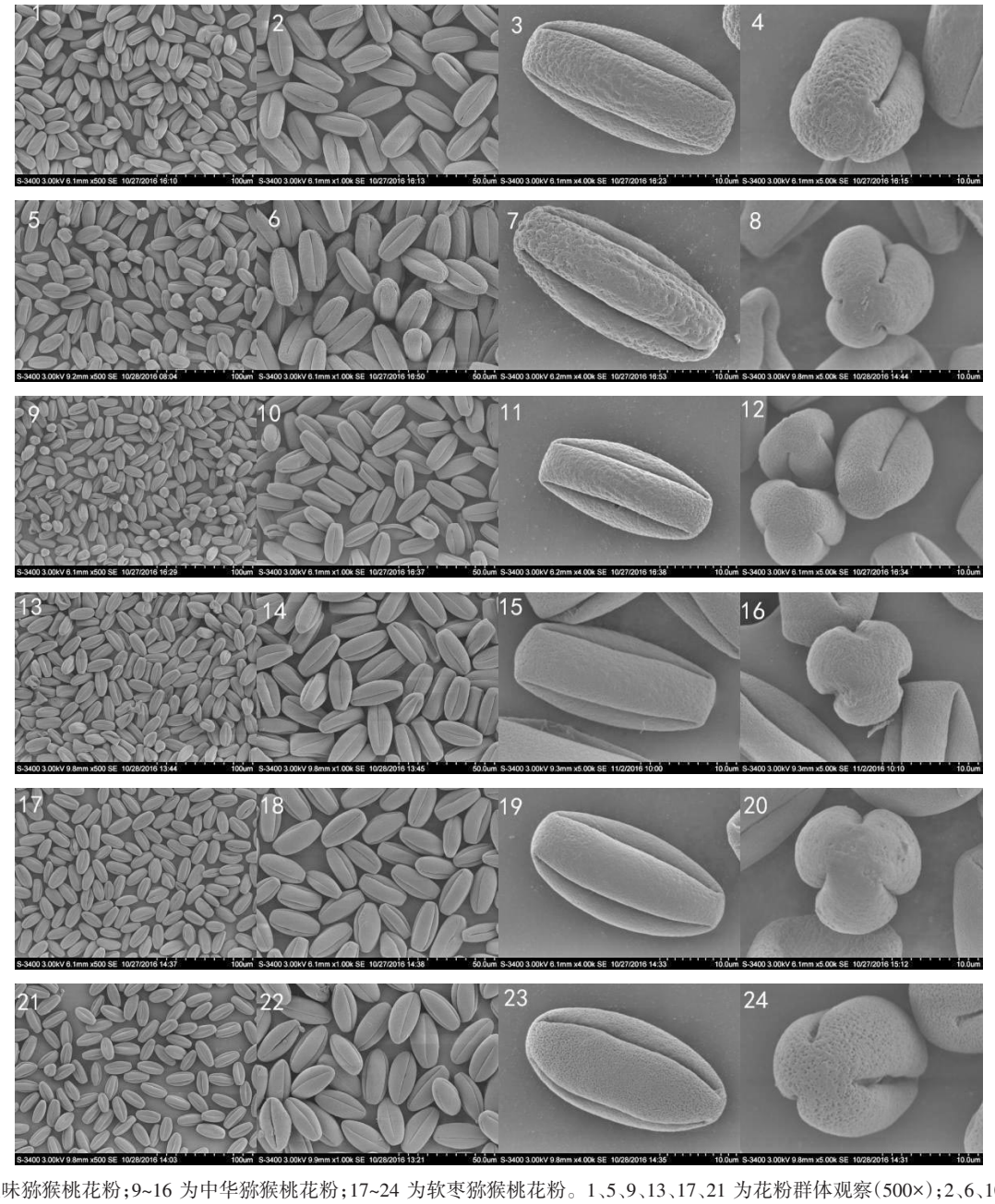
变异系数(*CV*)的计算：$CV/\%=s\times100/\bar{x}$，其中s为标准差。

2　结果与分析

2.1　花粉扫描电镜形态与大小

由图1可见，供试3个种的猕猴桃花粉均以单粒形式存在，外观形态呈长球形或超长球形，其中美味猕猴桃只有1份为长球形，其他均为超长球形；中华猕猴桃花粉均为超长球形；软枣猕猴桃10份供试材料中6份为超长球形、4份为长球形。所有花粉沿极轴方向都具有3条长行萌发沟，延伸至两极端，但在极区不形成合沟；从赤道面可观察到1~2条萌发沟，极面观均为三裂圆形，可看到3条内陷萌发沟。

表1统计了各单株花粉形态指标大小，存在差异。总体而言，萌发沟长度14.09~31.48 μm，平均23.13 μm，变异系数14.05%；萌发沟脊宽度4.09~11.42 μm，平均8.01 μm，变异系数16.81%；极轴长



1~8 为美味猕猴桃花粉;9~16 为中华猕猴桃花粉;17~24 为软枣猕猴桃花粉。1、5、9、13、17、21 为花粉群体观察(500×);2、6、10、14、18、22 为花粉群体观察(1 000×);3、7、11、15、19、23 为花粉赤道面观察(4 000×);4、8、12、16、20、24 为花粉极面观察(4 000×)。

1-8. *A. deliciosa* pollens; 9-16. *A. chinensis* pollens; 17-24. *A. arguta* pollens. 1, 5, 9, 13, 17, 21 are images captured of pollen grain under 500×; 2, 6, 10, 14, 18, 22 are images captured of pollen grain under 1 000×; 3, 7, 11, 15, 19, 23 are images captured of pollen grain in the equatorial view under 4 000×; 4, 8, 12, 16, 20, 24 are images captured of pollen grain in the polar view under 4 000×.

图 1 部分花粉扫描电镜形态

Fig. 1 SEM images of pollen morphology in *Actinidia* species

度 15.60~36.05 μm,平均 27.16 μm,变异系数 12.76%;赤道轴长度 8.76~18.42 μm,平均 13.12 μm,变异系数 13.34%;极轴/赤道轴 1.26~3.05,平均 2.08,变异系数 22.22%;大小 166.62~608.52 μm^2,平均 360.43 μm^2,变异系数 23.17%。供试花粉大小属于小到中等之间。相同种内,不同单株花粉形态指标之间存在差异。

2.2 花粉表面纹饰

由表 1 可见,供试猕猴桃的花粉外壁纹饰不尽相同,其扫描电镜图见图 2。(1)波纹状纹饰:表面较光滑,由不规则的条带状突起组成,轮廓线为细波浪形。16 份样品属于该种类型,占供试样品总数的

表1 供试猕猴桃花粉粒形态特征与大小

Table 1 The morphologic characteristics and size of pollen grain in *Actinidia* samples

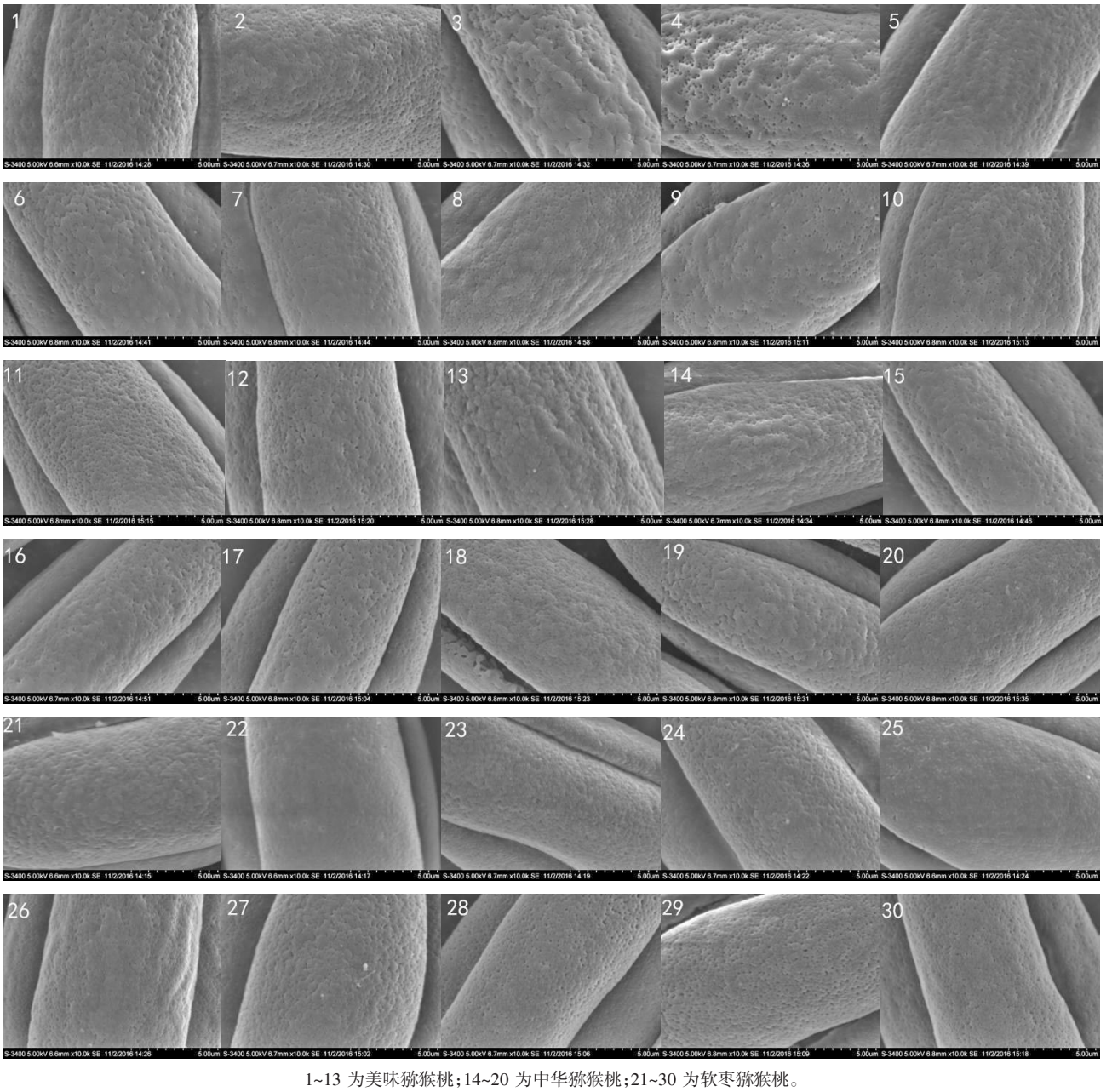
种 Species	序号 No.	代号 Code	来源 Origin	花粉形状 Pollen shape	极面观 Polar-view	萌发沟长度 Colpus length/μm	萌发沟脊宽度 Colpus width/μm	极轴长度 Polar axis length/μm	赤道轴长度 Equatorial axis length/μm	极轴/赤道轴 P/E shape	大小(极轴×赤道轴) Size (P×E)/μm²	表面纹饰 Surface ornamentation
美味猕猴桃 *A. deliciosa*	1	N12-27	米良1号实生 Seedlings of Miliang 1	超长球形 Perprolate	三裂圆形 Trifid circular	24.81±1.29 bcd	8.76±1.01 bc	28.41±1.53 bc	12.81±1.38 ab	2.23±0.23 c	364.56±50.91 b	疣状纹 Warty
	2	N3-4	米良1号实生 Seedlings of Miliang1	长球形 Prolate	三裂圆形 Trifid circular	26.64±1.80 d	9.02±1.15 c	28.18±2.19 b	14.44±1.22 ef	1.96±2.29 a	407.45±50.58 de	波纹状 Irregularly rugulate
	3	N6-20	米良1号实生 Seedlings of Miliang1	超长球形 Perprolate	三裂圆形 Trifid circular	23.42±2.56 ab	9.03±0.71 c	27.99±2.20 b	13.39±1.03 bc	2.10±0.19 b	375.53±47.87 bc	疣状纹 Warty
	4	N4-59	米良1号实生 Seedlings of Miliang1	超长球形 Perprolate	三裂圆形 Trifid circular	26.59±3.16 d	8.72±0.88 bc	31.64±1.81 f	14.45±1.25 ef	2.19±0.12 bc	459.45±48.45 gh	疣状纹 Warty
	5		猕宝♂(焦作) Mibao ♂(Jiaozuo, Henan)	超长球形 Perprolate	三裂圆形 Trifid circular	21.94±1.77 a	7.02±1.24 a	26.87±1.29 a	12.39±1.11 a	2.18±0.22 bc	333.27±35.97 a	疣状纹 Warty
	6	NS51-1	徐香×太行雄鹰2 Xuxiang×Taihangxiongying 2	超长球形 Perprolate	三裂圆形 Trifid circular	25.45±2.48 d	8.52±0.70 d	30.53±1.79 e	14.34±1.59 ef	2.15±0.24 bc	438.74±62.54 fg	波纹状 Irregularly rugulate
	7	NS51-26	徐香×太行雄鹰2 Xuxiang×Taihangxiongying 2	超长球形 Perprolate	三裂圆形 Trifid circular	23.87±1.94 d	8.42±0.52 cd	30.64±1.81 e	14.40±1.59 ef	2.15±0.24 bc	442.39±62.56 g	波纹状 Irregularly rugulate
	8	SSE5-4	秦美实生 Seedlings of Qinmei	超长球形 Perprolate	三裂圆形 Trifid circular	25.14±1.21 bcd	8.06±0.77 bc	29.80±1.28 de	13.83±1.13 cde	2.17±0.18 bc	412.75±43.29 def	疣状纹 Warty
	9	NS 4-66	米良1号实生 Seedlings of Miliang 1	超长球形 Perprolate	三裂圆形 Trifid circular	23.94±2.90 bc	8.43±1.11 bc	29.16±1.93 cd	13.58±1.37 cd	2.17±0.29 bc	395.80±45.01 cd	疣状纹 Warty
	10		金魁♂ Jinkui ♂	超长球形 Perprolate	三裂圆形 Trifid circular	25.97±2.61 cd	8.75±0.76 bc	31.84±1.99 f	14.83±1.26 f	2.16±0.16 bc	473.49±62.57 h	疣状纹 Warty
	11	SS5-4	海沃德实生 Seedlings of Hayward	超长球形 Perprolate	三裂圆形 Trifid circular	24.33±1.21 bc	7.86±1.07 ab	30.59±1.43 e	14.21±1.09 def	2.16±0.16 bc	435.06±46.47 efg	波纹状 Irregularly rugulate
	12	NS5,6-1	米良1号×太行雄鹰 Miliang1×Taihangxiongying	超长球形 Perprolate	三裂圆形 Trifid circular	25.74±1.29 cd	8.73±1.37 bc	30.39±1.92 e	14.57±0.92 f	2.09±0.16 b	443.09±43.51 g	波纹状 Irregularly rugulate
	13		海沃德实生 Seedlings of Hayward	超长球形 Perprolate	三裂圆形 Trifid circular	24.86±1.60 bcd	8.16±0.55 bc	30.06±1.29 de	13.64±1.06 cd	2.21±0.16 bc	410.40±41.87 de	疣状纹 Warty
中华猕猴桃 *A. chinensis*	14	N1-32	西峡野生中华混合果实实生 Seedlings of mixed fruits of *A. chinensis* from Xixia country, Henan province	超长球形 Perprolate	三裂圆形 Trifid circular	19.54±1.81 b	6.63±0.61 b	21.64±1.66 a	10.42±0.90 a	2.09±0.20 ab	225.90±30.60 a	疣状纹 Warty
	15	N13-5	西峡野生中华混合果实实生 Seedlings of mixed fruits of *A.chinensis* from Xixia country, Henan province	超长球形 Perprolate	三裂圆形 Trifid circular	18.98±1.34 ab	6.30±0.72 ab	23.18±1.16 b	10.92±1.47 a	2.15±0.21 b	253.73±41.67 b	波纹状 Irregularly rugulate
	16	S4-11	Hort-16A ♂	超长球形 Perprolate	三裂圆形 Trifid circular	23.29±1.71 c	7.70±0.97 c	26.70±1.70 c	12.17±0.82 b	2.20±0.18 b	325.16±33.38 c	波纹状 Irregularly rugulate
	17		金桃♂ Jintao ♂	超长球形 Perprolate	三裂圆形 Trifid circular	22.28±1.89 c	6.64±0.93 b	27.02±1.83 c	12.87±0.92 c	2.11±0.17 ab	348.17±39.49 d	波纹状 Irregularly rugulate

表1(续) Table 1(Continued)

种 Species	序号 No.	代号 Code	来源 Origin	花粉形状 Pollen shape	极面观 Polar-view	萌发沟长度 Colpus length/μm	萌发沟脊宽度 Colpus width/μm	极轴长度 Polar axis length/μm	赤道轴长度 Equatorial axis length/μm	极轴/赤道轴 P/E shape	大小(极轴×赤道轴) Size (P×E)/μm²	表面纹饰 Surface ornamentation
	18	N3-44	西峡野生中华混合果实实生 Seedlings of mixed fruits of *A.chinensis* from Xixia country, Henan province	超长球形 Perprolate	三裂圆形 Trifid circular	17.22±2.40 a	5.57±1.07 a	21.90±1.58 a	10.87±1.10 a	2.03±0.21 a	238.61±35.41 ab	波纹状 Irregularly rugulate
	19		薄山碧玉♂ Boshanbiyu♂	超长球形 Perprolate	三裂圆形 Trifid circular	17.51±2.01 a	6.46±0.88 b	21.69±1.15 a	10.81±1.05 a	2.02±0.18 a	234.92±29.33 ab	疣状纹 Warty
	20		红阳♂ Hongyang♂	超长球形 Perprolate	三裂圆形 Trifid circular	20.23±1.84 b	6.84±1.05 b	22.35±2.41 ab	10.69±0.92 a	2.10±0.26 ab	239.17±36.01 ab	波纹状 Irregularly rugulate
软枣猕猴桃 *A. argutar*	21	栾川 Luanchuan	栾川野生软枣混合果实实生 Seedlings of mixed fruits of *A. arguta* from Luanchuan country, Henan province	超长球形 Perprolate	三裂圆形 Trifid circular	21.10±3.18 a	7.70±0.73 a	24.10±2.33 a	12.04±1.76 a	2.02±0.19 c	293.09±65.12 a	波纹状 Irregularly rugulate
	22	N2-21	栾川野生软枣混合果实实生 Seedlings of mixed fruits of *A. arguta* from Luanchuan country, Henan province	超长球形 Perprolate	三裂圆形 Trifid circular	26.05±2.04 c	9.10±1.11 bcd	27.13±1.79 b	12.75±1.13 cd	2.14±0.18 d	346.78±44.53 cd	波纹状 Irregularly rugulate
	23	N2-34	栾川野生软枣混合果实实生 Seedlings of mixed fruits of *A. arguta* from Luanchuan country, Henan province	超长球形 Perprolate	三裂圆形 Trifid circular	23.65±1.87 b	8.34±1.45 ab	27.24±1.094 bc	13.22±1.23 d	2.08±0.18 cd	360.40±41.61 d	波纹状 Irregularly rugulate
	24	N10-52	栾川野生软枣混合果实实生 Seedlings of mixed fruits of *A. arguta* from Luanchuan country, Henan province	长球形 Prolate	三裂圆形 Trifid circular	22.10±1.73 ab	8.26±0.92 ab	25.03±2.80 d	14.33±1.53 d	1.76±0.26 a	359.43±61.60 d	波纹状 Irregularly rugulate
	25	N10-57	栾川野生软枣混合果实实生 Seedlings of mixed fruits of *A. arguta* from Luanchuan country, Henan province	超长球形 Perprolate	三裂圆形 Trifid circular	23.24±1.71 ab	8.67±0.67 bc	26.27±1.28 ab	12.63±1.29 bc	2.11±0.25 cd	332.43±38.25 bc	波纹状 Irregularly rugulate
	26	NS9-54	栾川野生软枣混合果实实生 Seedlings of mixed fruits of *A. arguta* from Luanchuan country, Henan province	长球形 Prolate	三裂圆形 Trifid circular	21.13±1.58 a	7.45±0.38 a	23.99±1.84 bc	13.13±1.12 ab	1.84±0.18 ab	315.57±42.51 ab	疣状纹 Warty
	27	N10-52	栾川野生软枣混合果实实生 Seedlings of mixed fruits of *A. arguta* from Luanchuan country, Henan province	长球形 Prolate	三裂圆形 Trifid circular	22.55±2.37 ab	8.37±0.75 ab	25.70±1.69 c	13.61±0.99 cd	1.90±0.14 b	350.39±41.31 cd	波纹状 Irregularly rugulate
	28		魁绿♂ Kuilü♂	超长球形 Perprolate	三裂圆形 Trifid circular	26.11±1.82 c	9.91±1.11 d	29.77±1.47 d	14.42±1.13 f	2.07±0.12 cd	430.36±49.93 f	颗粒状 Minutely granular
	29	SNE4-21	黑龙江野生软枣混合果实实生 Seedlings of mixed fruits of *A. arguta* from Heilongjiang province	超长球形 Perprolate	三裂圆形 Trifid circular	22.35±2.55 ab	7.43±0.71 a	27.54±1.34 c	13.49±1.10 de	2.05±0.16 cd	371.76±38.19 de	颗粒状 Minutely granular
	30	SNE5-12	黑龙江野生软枣混合果实实生 Seedlings of mixed fruits of *A. arguta* from Heilongjiang province	长球形 Prolate	三裂圆形 Trifid circular	22.29±2.40 ab	9.43±1.11 cd	27.48±1.44 d	14.36±0.92 e	1.91±0.12 b	395.07±40.19 e	颗粒状 Minutely granular

注:相同种内进行差异显著性分析,不同小写字母表示在 $P<0.05$ 差异显著。下同。

Note: Analysis of differences in pollen characteristics within the same *Actinidia* species. Means with different small letters indicate significant difference at $P<0.05$. The same below.



1~13 为美味猕猴桃;14~20 为中华猕猴桃;21~30 为软枣猕猴桃。

1−13. *A. deliciosa* pollens; 14−20. *A. chinensis* pollens; 21−30. *A. arguta* pollens.

图 2　30 份花粉的外壁纹饰特征(10 000×)

Fig. 2 SEM images of characteristics in pollen surface ornamentation (10 000×)

53.33%。其中美味猕猴桃样品占5份,占该种花粉总数的38.46%;中华猕猴桃占5份,占该种花粉总数的71.43%;软枣猕猴桃7份,占该种花粉总数的70%。(2)疣状纹饰:表面由规则或不规则的块状突起组成,大小不规则,轮廓线为不均匀的波浪形。11份样品属于该种类型,占供试样品总数的36.67%。其中美味猕猴桃样品8份,占该种花粉总数的61.54%;中华猕猴桃2份,占该种花粉总数的28.57%;软枣猕猴桃1份,占该种花粉总数的10%。(3)颗粒状纹饰:表面分布着颗粒突起或呈颗粒状的图案,轮廓线上常为微波浪型。3份样品属于该种类型,占供试样品总数的10.00%。均为软枣猕猴桃,占该种花粉总数的30%。

2.3　不同种类猕猴桃花粉粒形态特征与大小

表2统计了同种猕猴桃不同单株花粉粒形态特征与大小。(1)美味猕猴桃:萌发沟长度18.08~31.48 μm,变异系数9.59%;萌发沟脊宽度4.65~11.42 μm,变异系数12.40%;极轴长度21.54~36.05 μm,变异系数7.6%;赤道轴长度9.60~17.66 μm,变异系数10.01%;极轴/赤道轴1.65~3.05,变异系数9.68%;大

表 2 猕猴桃属 3 种猕猴桃花粉形态特征

Table 2 The morphological characteristics of pollen grain of three species in *Actinidia*

种 Species	萌发沟长度 Colpus length/μm	萌发沟脊宽度 Colpus width/μm	极轴长度 Polar axis length/μm	赤道轴长度 Equatorial axis length/μm	极轴/赤道轴 P/E shape	大小(极轴×赤道轴) Size(P×Es)/μm²
美味猕猴桃 *A. deliciosa*	24.94±2.39 c	8.42±1.04 b	29.70±2.24 c	13.91±1.39 c	2.15±0.21 a	414.77±62.46 c
中华猕猴桃 *A. chinensis*	19.87±2.78 a	6.59±1.05 a	23.50±2.75 a	11.25±1.33 a	2.10±0.21 b	266.52±57.35 a
软枣猕猴桃 *A. arguta*	23.06±2.68 b	8.47±1.20 b	26.43±2.43 b	13.40±1.45 b	1.99±0.22 a	355.53±59.438 b

小244.90~608.52 μm²,变异系数15.06%。(2)中华猕猴桃:萌发沟长度14.09~24.96 μm,变异系数14.00%;萌发沟脊宽度4.09~9.37 μm,变异系数15.88%;极轴长度15.60~30.11 μm,变异系数11.17%;赤道轴长度8.85~17.87 μm,变异系数11.80%;极轴/赤道轴1.26~2.65,变异系数9.93%;大小182.58~443.18 μm²,变异系数21.52%。(3)软枣猕猴桃:萌发沟长度16.34~30.06 μm,变异系数11.62%;萌发沟脊宽度6.11~11.23 μm,变异系数14.13%;极轴长度18.66~32.11 μm,变异系数9.21%;赤道轴长度8.76~18.42 μm,变异系数10.79%;极轴/赤道轴1.33~2.68,变异系数10.90%;大小166.62~547.07 μm²,变异系数16.70%。不同种间进行比较,中华猕猴桃的萌发沟长度、萌发沟脊宽度、极轴长度、赤道轴长度、大小(极轴×赤道轴)均表现最小,与美味猕猴桃和软枣猕猴桃差异显著;美味猕猴桃萌发沟长度、极轴长度、赤道轴长度、大小(极轴×赤道轴)均表现最大,与中华猕猴桃和软枣猕猴桃差异显著。

3 讨 论

本试验中,不同种猕猴桃形态均为长球形或超长球形,且萌发孔类型为三孔沟,极面观为三裂圆形,赤道面观察到1~2条萌发沟,沟长达两端,但不形成合沟,进一步证实了前人研究的猕猴桃属植物花粉在形状上变异较少[12]的结论。同时研究发现,供试的猕猴桃花粉不同种间在萌发沟长度、萌发沟脊宽度、极轴长度、赤道轴长度、极轴/赤道、花粉粒大小以及外壁纹饰等方面均有较大差异,种内个别性状也存在差异。同为'米良1号'实生后代的1、2、3、4、9号供试样品间以及同为栾川野生软枣混合果实实生后代的21~27号供试样品间多数性状均存在显著差异,呈现出了遗传多样性,可能与自然实生后代雄株的不一致有一定关联。同为'徐香'×'太行雄鹰2'的杂交F_1代6、7号供试花粉各性状没有显著差异,纹饰也表现一致,体现出了遗传的保守性。另外,相同种内不同单株花粉大小各性状的变异系数均小于所有样品之间比较的变异系数,可见相同种内花粉大小性状存在一定的保守性。物种花粉形态变化可能由基因型的差异造成[6,8]。

美味猕猴桃表面纹饰为疣状或波纹状,以前者为主。中华猕猴桃表面纹饰为疣状或波纹状,以后者为主。软枣猕猴桃3种纹饰均存在,可能与其自然分布广泛、遗传差异较大有关;软枣猕猴桃花粉纹饰主要以波纹状和颗粒状为主,其中3种颗粒状样品均来源于黑龙江,波纹状主要来源于栾川地区野生软枣猕猴桃混合果实实生。上述研究体现出猕猴桃属植物花粉表面纹饰具有丰富遗传多样性的同时,在相同种内和相同来源环境背景下存在一定的保守性。与钟敏等[9]研究的野生毛花猕猴桃在花粉的形态、外壁纹饰方面存在遗传保守性和多样性的结果一致。另外,美味猕猴桃、中华猕猴桃野生资源集中分布于长江流域中下游,而软枣猕猴桃资源在全国分布最为广泛,花粉粒纹饰的遗传多样性以及相同区域内的遗传保守性可能也与物种的进化有关。

笔者前期研究表明,美味猕猴桃花粉粒直径大于中华猕猴桃和软枣猕猴桃,但是由于供试的软枣猕猴桃样品较少,尽管供试的软枣猕猴桃花粉直径大于中华猕猴桃,但在该文中未做中华猕猴桃和软枣猕猴桃花粉大小比较的最终结论[10]。本研究中在各种类型花粉数量均较多的情况下,美味猕猴桃花粉仍然最大;整体来看,软枣猕猴桃花粉显著大于中华猕猴桃,从单个样品来看只有2份样品略小于中华猕猴桃样品。前人研究[4,6,13]认为花粉的大小与进化程度相关,花粉粒变小可以减少对树体营养的竞争,因而体积小的花粉为进化程度较高的类型。因此,美味猕猴桃进化程度在3种花粉中表现最低,同

时从表面纹饰特征来看,中华猕猴桃和美味猕猴桃均为波纹状或疣状。基于这点,支持“中华猕猴桃和美味猕猴桃2个种本身为遗传近缘种,2者具有极高的遗传相似性”[15]和“美味猕猴桃是从中华猕猴桃的变种提升成为一个独立种”[14]的推论。但是从本文研究的花粉大小、萌发沟长度、萌发沟脊宽度、极轴长度、赤道轴长度来看,中华猕猴桃均显著小于美味猕猴桃,可作为2者划分的孢粉学特征依据。当然,对于中华猕猴桃和美味猕猴桃的分类一直存在争议,孢粉学数据只是一个微观性状,与传统的形态分类特征可进行合理的结合,共同判断。对于软枣猕猴桃和中华猕猴桃进化程度的比较,从多数花粉大小上推断似乎应该中华猕猴桃进化程度高于软枣猕猴桃。另外,生产中一般美味猕猴桃整体上大于中华猕猴桃和软枣猕猴桃,但软枣猕猴桃的果实一般都小于中华猕猴桃,因此本研究结论与前人提出的“可以用物种雄株花粉粒的大小作为推断雌株果实大小指标”[6]的结论不尽相同,花粉大小与果实大小相关性的结论可能与这3个栽培种不一定吻合。所以,基于花粉形态学观察推测雌株果实大小以及研究系统发育有待于在更多种类及加大种内样品数量的前提下进一步研究才能确定。

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