

基于全基因组信息的常山胡柚遗传鉴定

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摘 要:【目的】常山胡柚是我国一个优良地方柑橘资源, 但对其遗传背景一直存在争议, 有必要进一步探究常山胡柚的遗传背景。【方法】本研究基于全基因组重测序数据结果, 并用分子标记进行验证, 同时结合形态特征以及果实品质数据, 将常山胡柚与相关柑橘资源进行系统比较。【结果】基因组重测序数据表明, 常山胡柚中超过 87.91% 的遗传组分为柚橘杂合, 柚纯合的遗传组分为 9.77%, 且全基因组序列与酸橙相似度较高, 其中与江津酸橙序列相似度达 88.34%, 表明常山胡柚是一种酸橙。根据不同位点开发的 4 对 InDel 分子标记进行检测, 也印证了常山胡柚与酸橙亲缘关系较近的结论。表型和品质数据表明, 常山胡柚在叶片形态上与酸橙相近, 且翼叶较小, 果实形态类似酸橙, 且单果质量、果皮厚度、种子质量、果皮质量等形态指标也与酸橙无显著性差异, 而与橘、柚及葡萄柚存在显著差异。常山胡柚可滴定酸含量、固酸比等品质指标较酸橙明显优化。【结论】常山胡柚为酸橙遗传背景, 是经长期栽培驯化后形成的一种果实品质改良的酸橙。

关键词: 常山胡柚; 酸橙; 基因组分析; 分子标记; 果实品质

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Genetic identification of Changshan Huyou based on whole genome information

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Abstract: 【Objective】Changshan Huyou is an excellent local citrus resource in China, but its genetic background is always controversial. The study aimed to explore the genetic background of Changshan Huyou. 【Methods】Changshan Huyou was used as the experimental material. Other tested materials included typical citrus species resources and local citrus resources in Zhejiang, such as mandarin orange, pummelo and sour orange. Firstly, the morphological characteristics of leaves and fruits of Changshan Huyou were compared with mandarin orange, pummelo, sour orange and grapefruit. Secondly, the fruit quality of different citrus resources was determined, the transverse and longitudinal diameter of fruit was measured by vernier caliper, and the fruit shape index was calculated, the weights of single fruit, peel, residue and seed were measured by electronic balance, and the edible rate was calculated, the soluble solids and titratable acids were measured by hand-held sugar acid instrument, and the solid acid ratio of fruit was calculated, the content of ascorbic acid was determined by 2,6-dichlorophenol indophenol reagent. The differences in appearance quality and internal quality of the various resources were analyzed. The genome of Changshan Huyou was resequenced, and the sweet orange was used as the reference genome. Changshan Huyou was compared with the sweet orange reference genome through gatk

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4.0 for SNP-calling, and finally the SNP data of Changshan Huyou, mandarin, sour orange, pummelo and grapefruit were obtained. The SNP information in each window was analyzed, so as to judge the genetic source of each window and obtain the genetic component map of their whole genome. Based on the genomic difference information of mandarin orange, pummelo, Changshan Huyou, sour orange and grapefruit, specific InDel molecular markers were developed, and Changshan Huyou was further identified by the molecular markers. **【Results】** The morphological analysis of leaves and fruits showed that there were great differences in leaf types of different citrus resources. The leaf morphology of Changshan Huyou was similar to that of sour orange, close to oval or ovate, and its wing leaves were smaller, while the wing leaves of pummelo were larger. Changshan Huyou belongs to small and medium-sized fruit type, which is similar in size to sour orange. The peel is golden yellow, the flesh is light yellow, and the peel thickness is similar to that of sour orange. The leaf type index of Changshan Huyou was not significantly different from that of Zigui sour orange, but significantly different from those of Bendizao mandarin orange, StarRuby grapefruit and Siji pummelo. The fruit quality data showed that Changshan Huyou had no significant difference from sour orange in appearance quality indexes such as single fruit weight, peel thickness, Seed weight, and peel weight, but had significant differences from mandarin orange, grapefruit and pummelo. There were significant differences between Changshan Huyou and sour orange in internal quality indexes such as titratable acid and solid acid ratio. The average content of titratable acid was 0.98%, and the average solid acid ratio was 12.30. The resequencing data showed that more than 87.91% of the genetic regions of Changshan Huyou were hybrid origin between pummelo and mandarin orange, and 9.77% of the genetic regions were homozygous to pummelo. By comparing with the whole genome structure of other tested citrus materials, it was found that the genome structure of Changshan Huyou was mostly similar to that of sour orange, and the sequence similarity with Jiangjin sour orange was 88.34%. By clustering Changshan Huyou with other citrus resources, it was also found that Changshan Huyou was clustered with sour orange, indicating that Changshan huyou would a kind of sour orange. In addition, four pairs of InDel molecular markers were developed according to the different variant ectopic sites of the whole genome, and they could be used to identify the genetic differences among mandarin orange, pummelo, Changshan Huyou, sour orange and grapefruit. Through the verification of molecular markers, it was found that mandarin orange had a long band, pummelo and grapefruit had a short band, sour orange had two bands of pummelo and mandarin, the amplified band size of Changshan Huyou was consistent with the sour orange band, and they were the two bands of mandarin orange and pummelo, which was inconsistent with pummelo and grapefruit, it was further confirmed that Changshan Huyou had a close genetic relationship with sour orange. **【Conclusion】** Based on the whole genome resequencing data, this study speculated the genetic background of Changshan Huyou, and systematically compared the molecular markers, morphological characteristics and fruit quality data of Changshan Huyou with the citrus resources related to Changshan Huyou. We conclude that 87.91% of the genetic components of Changshan Huyou are heterozygous, the whole genome sequence of Changshan Huyou is highly similar to that of sour orange, and it is also clustered with sour orange, which indicates that Changshan Huyou has the genetic background of sour orange. This is also confirmed by InDel molecular markers. The leaf and fruit morphology of Changshan Huyou is similar to that of sour orange, but the fruit quality has been significantly improved, indicating that Changshan Huyou is a kind of sour orange with improved fruit quality obtained through long-term cultivation and domestication.

Key words: Changshan Huyou; Sour orange; Genome analysis; Molecular marker; Fruit quality

常山胡柚 (*Citrus aurantium* ‘Changshan Huyou’) 也叫常山柚橙、金柚、衢枳壳, 是我国地方特色柑橘资源, 主产区为浙江省衢州市常山县。据资料记载, 常山胡柚已有上百年的栽培历史, 最早在常山县青石镇底铺村和澄潭村栽培^[1]。目前, 青石镇澄潭村胡家自然村还保存一棵树龄超过百年的实生胡柚树, 如今栽培的常山胡柚大多源自这棵常山胡柚“祖宗树”^[2]。常山胡柚现已成为常山县特色农业支柱产业, 是当地果农的重要收入来源^[3]。

有关常山胡柚的遗传背景, 前人有不少研究。蒋柏宏^[4]通过分析胡柚的植物学性状, 推测常山胡柚可能是柚和其他柑橘属的天然杂交种。蔡剑华等^[5]通过过氧化物同工酶酶谱法分析, 发现常山胡柚有7条过氧化物同工酶酶谱带, 而柚的5条酶谱带与常山胡柚其中5条完全一致, 多余的2条正好与甜橙相吻合, 表明常山胡柚可能是柚和甜橙杂交而来。陈力耕等^[6]利用RAPD分子标记技术对常山胡柚、椪柑、甜橙、衢橘和柚进行分子鉴定, 推测常山胡柚可能是先由柚与椪柑或衢橘自然杂交, 再与甜橙自然杂交而得到的天然杂种。陈士超等^[7]采用RAPD和ISSR分子标记技术对常山胡柚和常山当地柑橘资源进行鉴定, 结果表明常山胡柚可能是由柚、甜橙和其他柑橘属资源通过多重杂交而来。Xu等^[8]通过对常山胡柚的可能亲本材料进行ITS序列分析, 发现常山胡柚有2个不同序列的ITS1, 其中

ITS1A序列与柚完全一致, ITS1B序列与酸橙完全一致, 推测常山胡柚是由柚与酸橙杂交而来。上述通过不同手段对常山胡柚的遗传背景进行了研究报道, 然而对其遗传上的鉴定却报道较少。

随着现代测序技术的迅速发展, 通过全基因组信息可以全面而准确地鉴定材料之间的变异。Xu等^[9]完成对二倍体甜橙的测序组装, 揭示了二倍体甜橙是柚与橘杂交2次产生的杂种后代。Wu等^[10]完成对克里曼丁橘的测序, 揭示了柑橘的驯化史和柑橘起源。前人^[11-13]完成了高质量柚、野橘、甜橙以及野生柑橘资源的基因组测序, 为进行资源鉴定提供了丰富的基因组信息。

研究基于全基因组重测序信息, 结合分子标记与橘、酸橙、葡萄柚、柚形态特征和品质性状对常山胡柚进行遗传鉴定, 结果显示常山胡柚全基因组具有酸橙遗传背景, 形态学特征与酸橙相似, 果实品质较酸橙有明显改良, 推测是一种品质改良的酸橙。可为进一步对常山胡柚的遗传背景分析和种质资源的保护利用提供理论依据。

1 材料和方法

1.1 试验材料

常山胡柚于2020年7月采样自浙江省衢州市常山县青石镇澄潭村。其他供试材料包括典型的柑橘资源与浙江地区的地方性橘、柚和酸橙等柑橘资源, 具体详见表1。

表1 供试柑橘资源信息

Table 1 Tested citrus resources information

序号 Number	柑橘资源 Citrus resources	序号 Number	柑橘资源 Citrus resources	序号 Number	柑橘资源 Citrus resources
1	枸橼 Citron	8	朱栾红果 Zhuluan hongguo	15	朱栾酸橙 Zhuluan sour orange
2	本地早 Bendizao mandarin	9	枸头橙 Goutou cheng	16	星路比葡萄柚 StarRuby grapefruit
3	温州蜜柑 Satsuma mandarin	10	黄岩代代 Huangyan daidai	17	文旦柚 Wendan pummelo
4	常山胡柚 Changshan Huyou	11	凯亚城酸橙 Kaiyacheng sour orange	18	青田红柚 Qingtianhong pummelo
5	代代酸橙 Daidai sour orange	12	江津酸橙 Jiangjin sour orange	19	四季柚 Siji pummelo
6	秭归酸橙 Zigui sour orange	13	四川酸橙 Sichuan sour orange	20	琯溪蜜柚 Guanxi pummelo
7	小红橙 Xiaohong cheng	14	巴西酸橙 Brazil sour orange		

注: 枸头橙、凯亚城酸橙为浙江台州黄岩区地方酸橙品种。

Note: Goutou cheng and Kaiyacheng sour orange are local sour orange varieties in Huangyan District, Taizhou, Zhejiang Province.

1.2 形态学观测

比较橘、酸橙、常山胡柚、葡萄柚和柚叶片与果实形态学上的差异。选择发育正常树体的中上部枝条, 取完整的成熟叶片进行拍照比较, 利用游标卡尺

测量叶片长度和宽度, 并计算叶形指数, 记录翼叶, 通过IBM SPSS Statistics 20软件用于显著性差异分析。同样在果实成熟期选择完整果实进行拍照^[14], 比较不同柑橘类果实正面与果实切面。

1.3 果实品质测定

选取橘、酸橙、常山胡柚、葡萄柚和柚的成熟果实进行品质测定,通过游标卡尺测量果实横径、纵径,并计算果形指数;采用电子天平测定果实单果质量、果皮质量、果渣质量、种子质量等指标,并计算可食率;采用手持糖酸仪测定可溶性固形物和可滴定酸,计算果实固酸比;2,6-二氯酚靛酚试剂测定抗坏血酸含量。最后通过IBM SPSS Statistics 20软件用于显著性差异分析。

1.4 全基因组重测序分析

取常山胡柚新鲜叶片于研钵中使用液氮对其进行充分研磨,将研磨好的冻样送测序公司(北京诺禾致源科技股份有限公司)进行全基因组重测序,测序深度25.7层,共获得17.5 G数据量。以甜橙作为参考基因组^[9],通过BWA软件^[15]将重测序数据比对到甜橙参考基因组,使用gatk4.0^[16]进行SNP-calling,最终获得常山胡柚与橘、酸橙、葡萄柚和柚的SNP数据。利用本实验室前期已获得的可区分柑橘几大基本种的诊断型SNP位点^[11],对常山胡柚与橘、酸橙、葡萄柚和柚基因组以200 kb为窗口、100 kb为步长进行滑动,将全基因组划分为3390个区域,并对每

一个窗口内的SNP信息进行统计分析用以判断每个窗口的遗传来源,获得各自全基因组的遗传成分图,并基于群体重测序鉴定到的高质量SNP及甜橙基因组鉴定到的4 dTV位点,最终使用131 387个4 dTV位点构建聚类树。

1.5 DNA提取及InDel分子标记开发

通过CTAB法对柑橘叶片进行DNA提取^[17]。利用SNP-calling后的InDel数据寻找橘、柚、常山胡柚、酸橙和葡萄柚基因组差异信息,开发特异性InDel分子标记,经过多对引物筛选确定了4对InDel引物可用于验证橘、柚、常山胡柚、酸橙和葡萄柚之间的遗传差异(由武汉擎科生物公司合成),其InDel变异位点位于2号染色体和7号染色体上不同位点,引物序列详见表2。PCR扩增使用高保真酶(Phanta Max Super-Fidelity DNA Polymerase)进行,反应体系为20 μ L,各组分为2 \times phanta Max Buffer 10 μ L, Phanta Max 0.4 μ L, dNTP Mix 0.4 μ L, Forward primer 0.5 μ L, Reverse primer 0.5 μ L, DNA原液1 μ L (150 ng左右), ddH₂O 7.2 μ L。在T100 Thermal Cycler型号PCR仪中进行扩增,PCR扩增反应条件为预变性95 $^{\circ}$ C 3 min,变性95 $^{\circ}$ C 15 s,退火54 $^{\circ}$ C 15 s,

表2 鉴定常山胡柚与供试柑橘资源的InDel引物信息

Table 2 Identification of InDel primer information of Changshan Huyou and tested citrus resources

引物名称 Primer name	引物序列(5' - 3') Primer sequence(5' - 3')	引物名称 Primer name	引物序列(5' - 3') Primer sequence(5' - 3')
InD1-chr2-F	GGAATGCCTCGGATTGAA	InD3-chr2-F	TGAATCCCACTGAAGCCTAT
InD1-chr2-R	TAGCACCAGACAAGACCACTC	InD3-chr2-R	TCGGTCCTCTATCATCCTCT
InD2-chr7-F	AATGGCTCTGCTATGACGA	InD4-chr2-F	GGATTGATTGGTCCACTGCTC
InD2-chr7-R	GACGGATGGTGACCTAACAAAG	InD4-chr2-R	GCAAAGCGGGTTTAGTAATGAT

延伸72 $^{\circ}$ C 40 s, 35个循环,终延伸72 $^{\circ}$ C 5 min。PCR扩增产物在1.5%琼脂糖凝胶上进行电泳检测,电泳检测条件为恒定电压120 V、电流400 mA、额定功率100 W,电泳20 min,之后在Universal Hood II仪器中检测PCR产物。

2 结果与分析

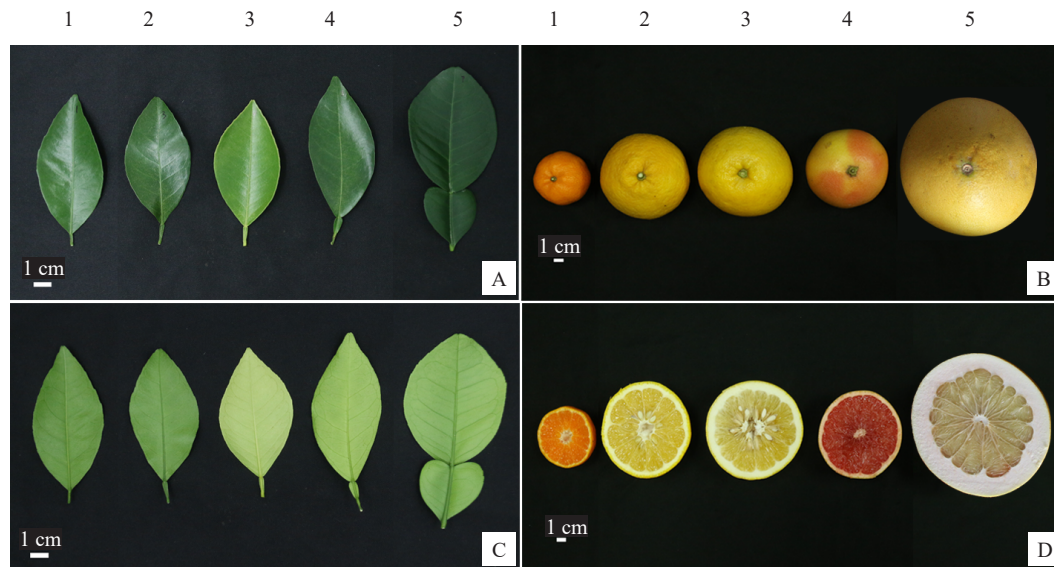
2.1 叶、果形态学分析

通过对本地早橘、秭归酸橙、常山胡柚、星路比葡萄柚和四季柚的叶片和果实进行观察(图1),不同柑橘资源的叶形差异较大,常山胡柚的叶片形态与酸橙相近,接近卵圆形或卵形,且翼叶较小,而柚翼叶较大。常山胡柚属于中小果型,大小

与酸橙相似,果皮金黄色,果肉呈淡黄色,果皮厚度与酸橙相近。另从叶形指数可以看出常山胡柚与秭归酸橙差异不显著,而与本地早橘、星路比葡萄柚和四季柚差异显著(表3)。

2.2 不同柑橘资源常规品质分析

选取本地早橘、秭归酸橙、星路比葡萄柚和琯溪蜜柚等典型柑橘品种与常山胡柚进行果实品质分析。由表4可知,不同柑橘种类的内外品质存在差异,常山胡柚在单果质量、果皮厚度、种子质量和果皮质量等外观品质指标上与酸橙无显著差异,与橘、葡萄柚和柚存在显著差异。而常山胡柚在可滴定酸和固酸比等内在品质指标上与酸橙存在显著差异,其可滴定酸平均含量为0.98%,固酸比平均可达12.30,相较



A. 正面叶形;B. 果实正面;C. 反面叶形;D. 果实切面;1~5. 本地早橘,秭归酸橙,常山胡柚,星路比葡萄柚,四季柚。

A. Frontal Leaf type;B. Fruit front;C. Reverse Leaf type;D. Fruit section;1-5. Bendizao mandarin, Zigui sour orange, Changshan Huyou, StarRuby grapefruit, Siji pummelo.

图 1 常山胡柚与其他 4 份资源叶、果形态比较

Fig. 1 Comparison of leaf types between changshan Huyou and other four citrus accessions

表 3 常山胡柚与其他 4 份柑橘资源叶形态数据

Table 3 Leaf type data of Changshan Huyou and other four citrus resources

名称 Name	叶片横径 Blade width/ mm	叶片纵径 Blade length/ mm	叶形指数 Leaf profile index	翼叶横径 Wing leaf width/mm	翼叶纵径 Wing leaf length/mm	翼叶指数 Airfoil profile index
本地早橘 Bendizao mandarin	41.58±0.99 c	88.93±3.90 bc	2.14±0.07 a			
秭归酸橙 Zigui sour orange	51.90±1.57 a	95.03±2.81 ab	1.83±0.05 b			
常山胡柚 Changshan Huyou	48.97±0.86 ab	90.40±1.64 bc	1.85±0.03 b			
星路比葡萄柚 StarRuby grapefruit	45.51±0.60 bc	101.40±1.31 a	2.23±0.04 a			
四季柚 Siji pummelo	51.56±2.78 a	82.40±3.07 c	1.62±0.13 c	27.78	28.40	1.02

注:不同小写字母表示差异显著($p < 0.05$)。下同。

Note: Different lowercase letters indicate significant difference at $p < 0.05$. The same below.

表 4 不同柑橘资源的果实品质

Table 4 Fruit quality of different citrus resources

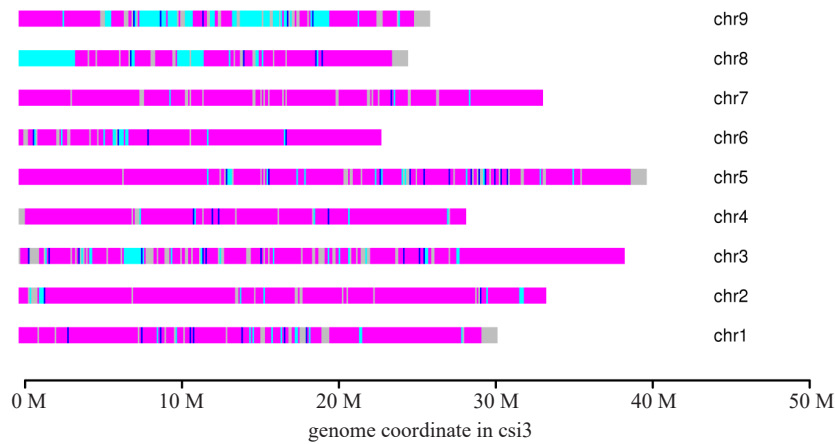
品质指标 Quality index	本地早橘 Bendizao mandarin	秭归酸橙 Zigui sour orange	常山胡柚 Changshan Huyou	星路比葡萄柚 StarRuby grapefruit	琯溪蜜柚 Guanxi pummelo
单果质量 Single fruit weigh/g	95.90±3.43 d	417.20±14.87 b	414.92±23.30 b	306.85±21.33 c	1 793.00±35.18 a
果形指数 Fruit type index	0.68±0.01 d	0.83±0.03 c	0.94±0.03 b	0.92±0.03 b	1.17±0.03 a
果皮厚度 Peel thickness/cm	0.28±0.02 d	0.69±0.12 bc	0.78±0.02 b	0.54±0.01 c	1.15±0.05 a
囊瓣数 Number of capsule valves	10.00±0.58 a	10.33±0.33 a	10.33±0.67 a	12.00±1.00 a	11.67±0.67 a
种子质量 Seed weight/g	0.61±0.54 bc	5.20±0.90 a	3.07±1.49 ab	0.23±0.12 c	0.00±0.00 c
果皮质量 Peel weight/g	26.44±3.90 d	105.27±11.81 bc	131.07±8.92 b	66.78±2.69 cd	582.09±25.15 a
w(可溶性固形物) Soluble solids content/%	11.30±0.76 ab	10.77±0.81 ab	12.04±0.39 ab	10.19±0.52 b	12.77±0.54 a
w(可滴定酸) Titratable acid content/%	0.60±0.04 c	3.21±0.43 a	0.98±0.04 c	2.16±0.21 b	0.72±0.06 c
固酸比 Solid acid ratio	19.10±1.99 a	3.42±0.29 c	12.30±0.70 b	4.79±0.43 c	17.83±1.08 a
w(抗坏血酸) Ascorbic acid content/(mg·100 g ⁻¹)	24.14±1.48 c	42.63±2.22 ab	47.67±3.47 a	36.05±0.60 b	43.76±1.92 a
可食率 Edible rate/%	72.13±2.74 ab	62.77±3.53 c	67.69±0.90 bc	78.07±0.83 a	69.06±0.88 bc

酸橙而言内在品质有明显改良。

2.3 胡柚全基因组分析

通过分析全基因组重测序数据,利用本实验室前期开发的柑橘基因组数据库,鉴定橘、常山胡柚、酸橙、葡萄柚和柚的遗传组成,结果显示常山胡柚全基因组超过87.91%的遗传组分为柚橘杂合,柚纯合

的遗传组分占9.77%(图2)。通过与其他供试柑橘材料的全基因组结构进行比较,发现常山胡柚与酸橙的基因组结构最为相似(图3),其中与江津酸橙的基因组结构相似度最高,达到了88.34%。通过常山胡柚与其他柑橘材料进行聚类,显示常山胡柚与酸橙聚类在一起(图4),综上表明常山胡柚是一种

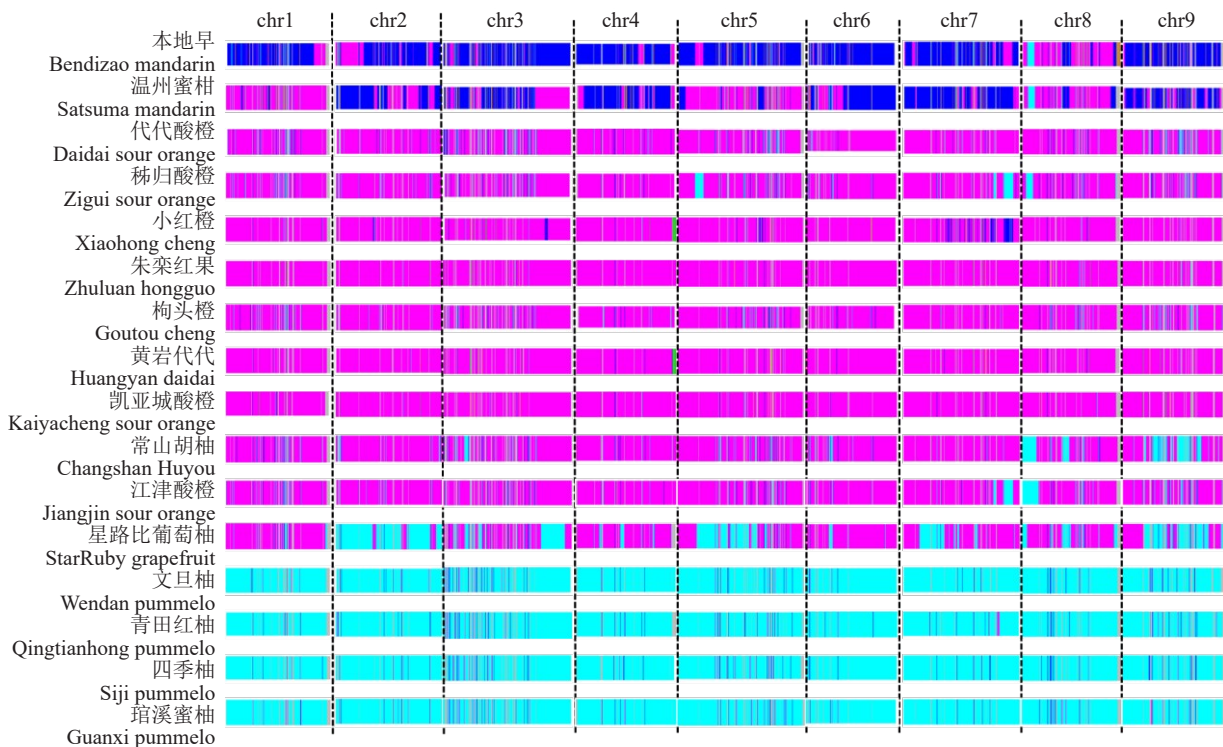


粉色 mp 区域 . 柚橘杂合;青色 pp 区域 . 柚;蓝色 mm 区域 . 橘。

Pink mp area. Hybrid of pummelo and mandarin; Cyan pp area. Pummelo; Blue mm area. Mandarin.

图 2 常山胡柚全基因组特征

Fig. 2 Characteristic map of whole genome of Changshan Huyou



粉色 mp 区域 . 柚橘杂合;青色 pp 区域 . 柚;蓝色 mm 区域 . 橘。

Pink mp area. Hybrid of pummelo and mandarin; Cyan pp area. Pummelo; Blue mm area. Mandarin.

图 3 常山胡柚与其他柑橘材料全基因组特征比较分析

Fig. 3 Comparative analysis of whole genome characteristics between Changshan Huyou and other citrus materials

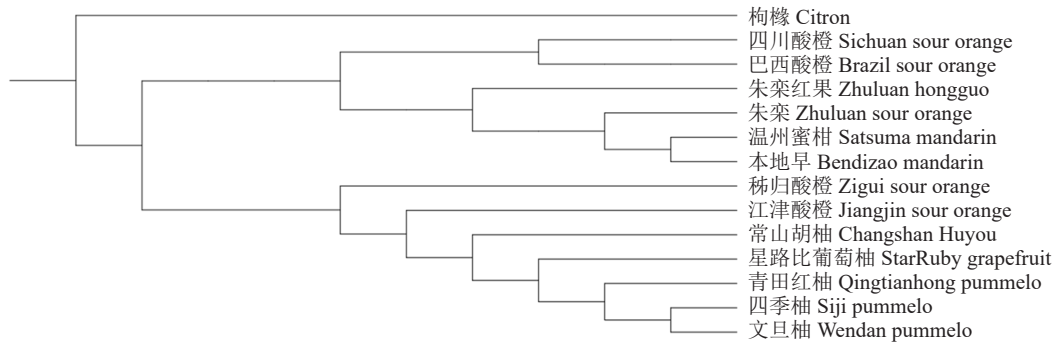


图4 常山胡柚与其他柑橘材料的聚类分析

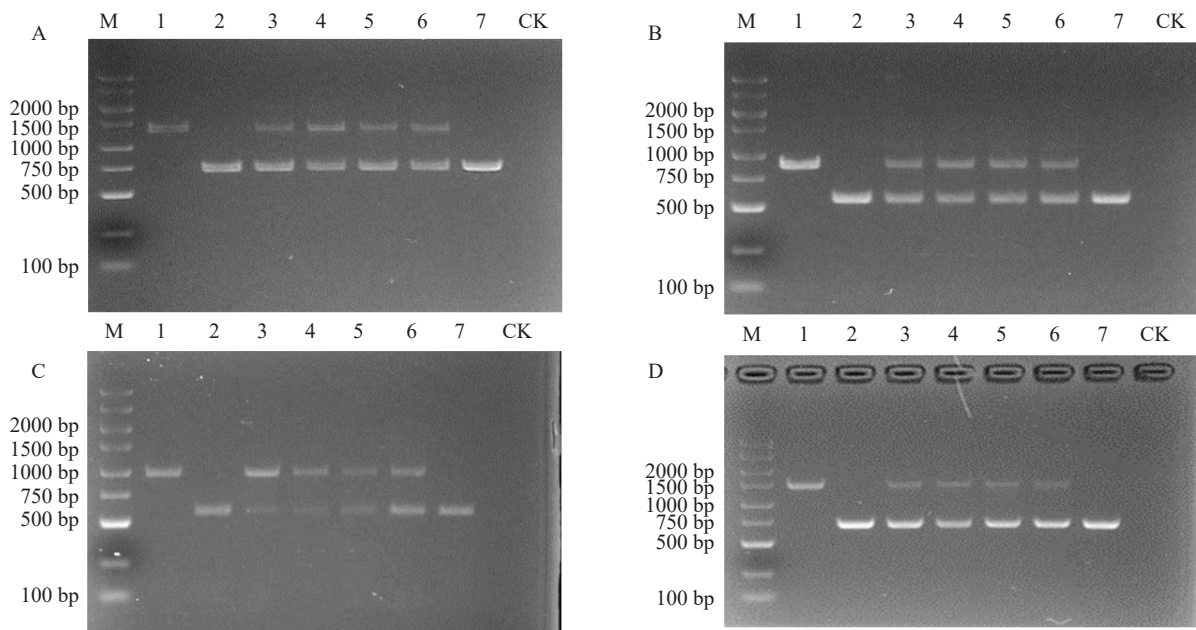
Fig. 4 Cluster analysis of Changshan Huyou and other citrus accessions

酸橙。

2.4 分子标记验证

通过前期分子标记的开发,在全基因组不同变异位点上筛选到4对 InDel 引物可用于鉴定橘、柚、

常山胡柚、酸橙与葡萄柚之间的遗传差异。结果如图5所示,橘为1条长带,柚和葡萄柚为1条短带,酸橙为柚和橘的2条带,常山胡柚扩增的条带大小与酸橙条带相符,为柚和橘的2条带,与柚和葡萄柚不



A. InD1-chr2; B. InD2-chr7; C. InD3-chr2; D. InD4-chr2; M. DNA 5 000 marker; 1~7. 本地早橘, 琯溪蜜柚, 常山胡柚, 凯亚城酸橙, 枸头橙, 秭归酸橙, 星路比葡萄柚; CK. 阴性对照。

A. InD1-chr2; B. InD2-chr7; C. InD3-chr2; D. InD4-chr2; M. DNA 5 000 marker; 1-7. Bendizao mandarin, Guanxi pummelo, Changshan huyou, Kaiyacheng sour orange, Goutou cheng, Zigui sour orange, StarRuby grapefruit; CK. Negative control.

图5 InDel 引物对常山胡柚与供试柑橘资源的凝胶电泳

Fig. 5 Gel electrophoresis of InDel primers on Changshan Huyou and tested citrus resources

符,故推测常山胡柚的亲缘关系与酸橙相近。

3 讨论

笔者在本研究中基于全基因组重测序技术,结合形态学与果实品质分析,发现常山胡柚全基因组信息表现为酸橙的特征,叶形态和果实大小与酸橙

相似,分子标记也印证与酸橙亲缘关系较近,表明常山胡柚是一种酸橙,且常山胡柚果实品质优于普通酸橙,其可滴定酸含量显著低于酸橙,是一种品质得到改良的酸橙,推测其在栽培过程中逐渐驯化为一种低酸类型的酸橙。

近年来,随着测序技术的快速发展,全基因组序

列为进一步探索物种进化和资源鉴定提供了更为全面的信息^[18-19]。笔者通过分析全基因组重测序数据,发现常山胡柚超过 87.91%的遗传组分为柚橘杂合成分,且常山胡柚的全基因组序列与酸橙的相似度较高,最高可达 88.34%,聚类分析显示常山胡柚与酸橙聚类在一起,表明常山胡柚是一种酸橙,符合常山胡柚 ITS1B 序列与酸橙完全一致的报道^[8]。而对于酸橙的亲本,目前普遍认为酸橙是柚与宽皮橘的 F₁ 代后代,且柚很可能是母本。该观点从同工酶、细胞学以及分子标记等多个方面得到证实^[20-22]。相关研究也验证了酸橙起源的模式,何发^[23]通过 SSR 分子标记验证酸橙起源模式为柚×橘,Wu 等^[24]从多份柑橘属资源的全基因组数据进行分析证实了酸橙的亲本为柚和宽皮橘。常山胡柚作为一种酸橙,推测其亲本为柚和橘,与前人报道常山胡柚的起源过程中柚和宽皮柑橘可能是主要的起源亲本相吻合^[6]。

分子标记技术自 20 世纪 80 年代开始广泛应用,也常常应用于果树种质资源鉴定与遗传分类^[25-28]。InDel 标记作为一种新型分子标记,适用于分析近缘物种间的基因组上同一位点序列插入和缺失的差异,且 InDel 标记开发成本较低、准确性高、多态性强和检测简便,因此受到广泛认可和应用^[29-30]。本研究对橘、柚、常山胡柚、酸橙和葡萄柚基因组重测序数据进行筛选,开发特异性 InDel 分子标记进一步印证常山胡柚的亲缘关系与酸橙相近,而非柚与葡萄柚。此外,在今后的研究中,也有望通过 InDel 标记应用于常山胡柚的精准育种中,为育种实践提供理论依据。

枳壳作为常用的大宗药材,如湘枳壳、川枳壳、江枳壳等都是传统中药材^[31],其次生代谢物常用于药理研究^[32],大部分地区标准均收载酸橙及其栽培变种作为枳壳的基原植物,如新疆、云南、香港等^[33]。常山胡柚未成熟果实可直接入药,自 2016 年以“衢枳壳”载入《浙江省中药炮制规范》后命名为衢枳壳,正式成为一味中药材^[34],表明常山胡柚为酸橙或酸橙的栽培种,与本研究鉴定常山胡柚是一种酸橙的结论相符,另本研究也可为常山胡柚用于中药材开发与应用提供遗传依据。

4 结 论

通过对常山胡柚进行形态学、果实品质、基因组重测序分析与 InDel 分子标记验证,初步明确常山胡柚为酸橙遗传背景,推测是经长期栽培驯化后形

成的一种果实品质改良的酸橙。这对柑橘资源的分类鉴定提供参考,为进一步探究常山胡柚的遗传起源进化提供依据,同时开发的 InDel 标记也有望应用于柑橘育种材料鉴定,为育种实践提供理论依据。

参考文献 References:

- [1] 俞日梁,陈新建,叶杏元,杨兴良,贝增明,郑国民. 常山胡柚品种起源及栽培研究进展[J]. 浙江林业科技, 2006, 26(3): 83-86. YU Riliang, CHEN Xinjian, YE Xingyuan, YANG Xingliang, BEI Zengming, ZHENG Guomin. Research progress on variety origin and cultivation of Changshan Huyou[J]. Journal of Zhejiang Forestry Science and Technology, 2006, 26(3): 83-86.
- [2] 张泽群. ‘红肉胡柚’起源及特征分析[D]. 杭州: 浙江农林大学, 2019. ZHANG Zequn. Analysis on the origin and characteristics of ‘Hongrou huyou’ [D]. Hangzhou: Zhejiang Agriculture and Forestry University, 2019.
- [3] 杨波,戴翔,汪丽霞,张志慧. 常山胡柚品牌管理和建设概述[J]. 浙江柑橘, 2021, 38(1): 8-11. YANG Bo, DAI Xiang, WANG Lixia, ZHANG Zhihui. Overview of Changshan Huyou brand management and construction[J]. Zhejiang Ganju, 2021, 38(1): 8-11.
- [4] 蒋柏宏. 常山胡柚的若干特性[J]. 浙江柑桔, 1994(2): 14-15. JIANG Baihong. Some characteristics of Changshan huyou[J]. Zhejiang Ganju, 1994(2): 14-15.
- [5] 蔡剑华,贺善安,缪天纲,叶杏元. 珍贵的柑桔种质资源: 金柚(胡柚)的研究[C]. 北京: 全国果树种质资源学术讨论会, 1995. CAI Jianhua, HE Shan'an, MIAO Tiangang, YE Xingyuan. Study on precious citrus germplasm resources: Jinyou (Huyou)[C]. Beijing: China Agricultural Science and Technology Press, 1995.
- [6] 陈力耕,胡西琴,赵四清. 关于胡柚起源的分子研究[J]. 园艺学报, 2002, 29(3): 276-277. CHEN Ligeng, HU Xiqin, ZHAO Siqing. Molecular study on the origin of Huyou[J]. Acta Horticulturae Sinica, 2002, 29(3): 276-277.
- [7] 陈士超,杨红,郑勇平,陈跃磊,邱英雄. 分子标记鉴定常山胡柚优良基因型的初步研究(英文)[J]. 分子细胞生物学报, 2006, 39(6): 502-508. CHEN Shichao, YANG Hong, ZHENG Yongping, CHEN Yuelei, QIU Yingxiong. Preliminary study on molecular marker identification of excellent genotypes of Changshan huyou (English)[J]. Journal of Molecular Cell Biology, 2006, 39(6): 502-508.
- [8] XU C J, BAO L, ZHANG B, BEI Z M, YE X Y, ZHANG S L, CHEN K S. Parentage analysis of huyou (*Citrus changshanensis*) based on internal transcribed spacer sequences[J]. Plant Breeding, 2010, 125(5): 519-522.
- [9] XU Q, CHEN L L, RUAN X A, CHEN D J, ZHU A S, CHEN C L, BERTRANG D, JIAO W B, HAO B H, LYON M P, CHEN J J, GAO S, XING F, LAN H, CHANG J W, GE X H, LEI Y, HU

- Q, MIAO Y, WANG L, XIAO S X, BISWAS M K, ZENG W F, GUO F, CAO H B, YANG X M, XU X W, CHENG Y J, XU J, LIU J H, LUO O J, TANG Z H, GUO W W, KUANG H H, ZHANG H Y, ROOSE M L, NAGARAJAN N, DENG X X, RUAN Y J. The draft genome of sweet orange (*Citrus sinensis*)[J]. *Nature Genetics*, 2013, 45(1):59-66.
- [10] WU G A, PROCHNIK S, JENKINS J, SALSE J, HELLSTEN U, MURAT F, PERRIER X, RUIZ M, SCALABRIN S, TEROL J, TAKITA M A, LABADIE K, POULAIN J, COULOUX A, JABBARI K, CATTONARO F, DEL F C, PINOSIO S, ZUCCOLO A, CHAPMAN J, GRIMWOOD J, TADEO F R, ESTORNELL L H, MUNOZ-SANZ J V, IBANEZ V, HERRERO-ORTEGA A, ALEZA P, PEREZ-PEREZ J, RAMON D, BRUNEL D, LURO F, CHEN C X, FARMERIE W G, DESANY B, KODIRA C, MOHIUDDIN M, HARKINS T, FREDRIKSON K, BURNS P, LOMSADZE A, BORODOVSKY M, REFORGIATO G, FREITAS-ASTUA J, QUETIER F, NAVARRO L, ROOSE M, WINCKER P, SCHMUTZ J, MORGANTE M, MACHADO M A, TALON M, JAILLON O, OLLITRAULT P, GMITTER F, ROKHSAR D. Sequencing of diverse mandarin, pummelo and orange genomes reveals complex history of admixture during citrus domestication[J]. *Nature Biotechnology*, 2014, 32(7):656-662.
- [11] WANG L, HE F, HUANG Y, HE J X, YANG S Z, ZENG J W, DENG C L, JIANG X L, FENG Y W, WEN S H, XU R W, YU H W, YANG X M, ZHONG G Y, CHEN C W, YAN X, ZHOU C F, ZHANG H Y, XIE Z Z, ROBERT M L, DENG X X, XU Q. Genome of wild mandarin and domestication history of mandarin[J]. *Molecular Plant*, 2018, 11(8):1024-1037.
- [12] WANG L, HE F, HUANG Y, HE J X, YANG S Z, ZENG J W, DENG C L, JIANG X L, FANG Y W, WEN S H, XU R W, YU H W, YANG X M, ZHONG G Y, CHEN C W, YAN X, ZHOU C F, ZHANG H Y, ZHANG H Y, XIE Z Z, BUCKLER E S, DENG X X, XU Q. Somatic variations led to the selection of acidic and acidless orange cultivars[J]. *Nature Plants*, 2021, 7(7):954-965.
- [13] WANG X, XU Y T, ZHANG S Q, CAO L, HUANG Y, CHENG J F, WU G Z, TIAN S L, CHEN C L, LIU Y, YU H W, YANG X M, LAN H, WANG N, WANG L, XU J D, JIANG X L, XIE Z Z, TAN M L, LARKIN R M, CHEN L L, MA B G, RUAN Y J, DENG X X, XU Q. Genomic analyses of primitive, wild and cultivated citrus provide insights into asexual reproduction[J]. *Nature Genetics*, 2017, 49(5):765-772.
- [14] 李蒙蒙. ‘抛橘’的遗传鉴定及其无核机理初步研究[D]. 武汉:华中农业大学, 2017.
- LI Mengmeng. Genetic identification of ‘Paoju’ and preliminary study on its seedless mechanism[D]. Wuhan: Huazhong Agricultural University, 2017.
- [15] LI H, DURBIN R. Fast and accurate short read alignment with burrows-wheeler transform[J]. *Bioinformatics*, 2009, 25(14):1754-1760.
- [16] MCKENNA A, HANNA M, BANKS E, SIVACHENKO A, CIBULSKIS K, KERNYTSKY A, GARIMELLA K, ALTSHULER D, GABRIEL S, DALY M, DEPRISTO M A. The genome analysis toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data[J]. *Genome Research*, 2010, 20(9):1297-1303.
- [17] 程运江. 柑橘体细胞胞质遗传及叶绿体 SSR 引物开发研究[D]. 武汉:华中农业大学, 2004.
- CHENG Yunjiang. Somatic cytoplasmic inheritance and development of chloroplast SSR primers in *Citrus*[D]. Wuhan: Huazhong Agricultural University, 2004.
- [18] 黄威剑, 李梦. 果树全基因组测序现状与展望[J]. *园艺学报*, 2021, 48(4):733-748.
- HUANG Weijian, LI Meng. Present situation and prospect of whole genome sequencing of fruit trees[J]. *Acta Horticulturae Sinica*, 2021, 48(4):733-748.
- [19] 彭洁, 余歆, 朱延松, 杨胜男, 程莉, 李仁静, 江东. 沃柑辐射诱变系渝沃无核的果实品质及遗传差异分析[J]. *果树学报*, 2021, 38(12):2119-2134.
- PENG Jie, YU Xin, ZHU Yansong, YANG Shengnan, CHENG Li, LI Renjing, JIANG Dong. Analysis of fruit quality and genetic difference of orah mutant Yuwowuhe derived from radiation[J]. *Journal of Fruit Science*, 2021, 38(12):2119-2134.
- [20] 方德秋, 章文才. 同工酶研究在柑橘上的应用[J]. *湖北农业科学*, 1990(7):22-24.
- FANG Deqiu, ZHANG Wencai. Application of isozyme research in citrus[J]. *Hubei Agricultural Sciences*, 1990(7):22-24.
- [21] 梁国鲁. 柑橘类的细胞分类学研究[J]. *武汉植物学研究*, 1990(8):1-7.
- LIANG Guolu. Cytotaxonomy of citrus[J]. *Wuhan Botanical Research*, 1990(8):1-7.
- [22] 李小孟. 柑橘及其近缘属植物的分子进化与栽培柑橘的起源研究[D]. 重庆:西南大学, 2010.
- LI Xiaomeng. Molecular evolution of citrus and its related genera and origin of cultivated citrus[D]. Chongqing: Southwest University, 2010.
- [23] 何发. 酸橙与甜橙的遗传起源及柑橘糖酸变化初探[D]. 武汉:华中农业大学, 2017.
- HE Fa. Genetic origin of sour orange and sweet orange and changes of citrus sugar and acid [D]. Wuhan: Huazhong Agricultural University, 2017.
- [24] WU G A, TEROL J, IBANEZ V, LÓPEZ-GARCÍA A, PÉREZ-ROMÁN E, BORREDÁ C, DOMINGO C, TADEO F R, CABBALLERO J C, ALONSO R, CURK F, DU D L, OLLITRAULT P, ROOSE M L, DOPAZO J, GMITTER F G, ROKHSAR D S, TALON M. Genomics of the origin and evolution of citrus[J]. *Nature*, 2018, 554(7692):311-316.
- [25] 葛聪聪, 王亚娜, 李鸾翔, 张艺琴, 袁小迈, 黄桂香. 香水柠檬×白花柠檬群体子代表型及遗传变异分析[J]. *果树学报*, 2022,

- 39(2):169-183.
- GE Congcong, WANG Yana, LI Luanxiang, ZHANG Yiqin, YUAN Xiaomai, HUANG Guixiang. Phenotypic and genetic variation of hybrid progenies of *Xiangshui lemon* × *Baihua lemon*[J]. Journal of Fruit Science, 2022, 39 (2):169-183.
- [26] 陈薇薇. 分子标记技术及其在果树种质资源上的应用分析[J]. 南方农业, 2018, 12(36): 139-140.
- Chen Weiwei. Molecular marker technology and its application in fruit germplasm resources[J]. South China Agriculture, 2018, 12(36):139-140.
- [27] 杜晓云,张青林,罗正荣. 逆转座子分子标记及其在果树上的应用[J]. 果树学报, 2009, 26(6):865-870.
- DU Xiaoyun, ZHANG Qinglin, LUO Zhengrong. Retrotransposon-based molecular markers and their applications in fruit trees[J]. Journal of Fruit Science, 2009, 26(6):865-870.
- [28] 沈向,郭卫东,任小林,李嘉瑞,郑学勤. 用 RAPD 再探核果类果树间亲缘关系[J]. 西北农业大学学报, 1999, 27(4):22-25.
- SHEN Xiang, GUO Weidong, REN Xiaolin, LI Jiarui, ZHENG Xueqin. Re-exploration of the genetic relationship among drupe fruit trees by RAPD[J]. Acta Universitatis Agriculturae Boreali-Occidentalis, 1999, 27(4):22-25.
- [29] 刘晨. 发掘长片段 InDel 变异精细定位大豆株高相关 *qPH16* 位点[D]. 大连: 辽宁师范大学, 2021.
- LIU Chen. Discovering long segment InDel variation and fine mapping *qPH16* loci related to soybean plant height[D]. Dalian: Liaoning Normal University, 2021.
- [30] 卢霞,刘梦华,邓志军,孙秋月,夏迎春,李文虎,司龙亭,阿门. 基于 InDel 标记的黄瓜种质资源遗传多样性分析[J]. 江苏农业科学, 2021, 49(1):49-54.
- LU Xia, LIU Menghua, DENG Zhijun, SUN Qiuyue, XIA Yingchun, LI Wenhui, SI Longting, Amen. Genetic diversity analysis of cucumber germplasm resources based on InDel markers[J]. Jiangsu Agricultural Sciences, 2021, 49(1):49-54.
- [31] 赵维良,郭增喜,张文婷,黄琴伟,依泽,宋剑锋. 药材枳壳基原植物种类及地理分布研究[J]. 中国中药杂志, 2018, 43(21): 4361-4364.
- ZHAO Weiliang, GUO Zengxi, ZHANG Wenting, HUANG Qinwei, YI Ze, SONG Jianfeng. Study on original plant species and geographical distribution of *Fructus Aurantii*[J]. China Journal of Chinese Materia Medica, 2018, 43(21):4361-4364.
- [32] 赵佳琛,王艺涵,翁倩倩,金艳,张卫,彭华胜,蔡秋杰,李兵,杨洪军,张华敏,詹志来. 经典方中枳实与枳壳的本草考证[J]. 中国现代中药, 2020, 22(8):1175-1184.
- ZHAO Jiachen, WANG Yihan, WENG Qianqian, JIN Yan, ZHANG Wei, PENG Huasheng, CAI Qiujie, LI Bing, YANG Hongjun, ZHANG Huamin, ZHAN Zhilai. Herbal textual research on *Fructus Aurantii immaturus* and *Fructus Aurantii immaturus* in classic famous prescriptions[J]. Modern Chinese Medicine, 2020, 22(8):1175-1184.
- [33] 林瑞超. 中国药材标准名录[M]. 北京: 科学出版社, 2011.
- LIN Ruichao. Standard list of Chinese medicinal materials[M]. Beijing: Science Press, 2011.
- [34] 杨波,汪丽霞,王满姬. 常山胡柚枳壳产业发展概况[J]. 浙江柑橘, 2019, 36(2):7-9.
- YANG Bo, WANG Lixia, WANG Manji. Development of Quzhiqiao briers industry[J]. Zhejiang Ganju, 2019, 36(2):7-9.