

基于全基因组重测序和叶绿体DNA序列的河北魏县梨地方品种资源分析

齐丹¹, 董星光^{1*}, 李振江^{2*}, 曹玉芬¹, 路露², 田路明¹, 李军喜²,
张莹¹, 霍宏亮¹, 苗秀晨², 徐家玉¹, 刘超¹, 李金岭², 朱蕊颖²

(¹中国农业科学院果树研究所·农业农村部园艺作物种质资源利用重点实验室,
辽宁兴城 125100; ²魏县林果开发服务中心, 河北魏县 056800)

摘要:【目的】解析河北魏县梨地方品种的遗传背景, 探讨魏县梨种质间的亲缘关系。【方法】以20个河北省魏县梨地方品种以及10个河北其他地区代表性梨地方品种为研究试材, 基于全基因组重测序数据和叶绿体DNA变异信息开展河北魏县梨地方品种的分子鉴定。【结果】魏县梨地方品种具有明显的群体遗传结构, 可分为两大类: 类群1由魏县红梨等9份材料组成, 与南方梨种群亲缘关系较近, 类群2由小白面等11份材料组成, 更接近北方梨种群。叶绿体DNA非编码区域的测序结果显示, 在魏县梨地方品种中检测到3种较进化的单倍型, 大部分地方品种的单倍型为H_3, 魏县红梨的单倍型为H_2, 雪花以及除金丰鸭梨外的鸭梨品种群的单倍型为H_1。分析品种间的亲缘关系和单倍型类别, 鉴定金丰鸭梨并非鸭梨的芽变品种, 可能为鸭梨的实生后代选育而成。白雪花不是雪花芽变, 白红梨不是魏县红梨的芽变, 油秋与武安油秋两者的遗传背景存在显著差异, 证实白砧子梨是砧子梨的芽变选育品种。【结论】明确魏县梨地方品种的亲缘关系和遗传背景, 为魏县地方梨种质资源的保护和高效利用提供理论基础。

关键词: 魏县梨; 全基因组重测序; 叶绿体DNA; 亲缘关系

中图分类号: S661.2

文献标志码: A

文章编号: 1009-9980(2025)04-0707-14

Genetic analysis of local pear varieties in Weixian county, Hebei province based on whole-genome resequencing and chloroplast DNA sequences

QI Dan¹, DONG Xingguang^{1*}, LI Zhenjiang^{2*}, CAO Yufen¹, LU Lu², TIAN Luming¹, LI Junxi², ZHANG Ying¹, HUO Hongliang¹, MIAO Xiuchen², XU Jiayu¹, LIU Chao¹, LI Jinling², ZHU Ruiying²

(¹Research Institute of Pomology, Chinese Academy of Agricultural Sciences/Key Laboratory of Biology and Genetic Improvement of Horticultural Crops Germplasm Resources Utilization, Ministry of Agriculture and Rural Affairs, Xingcheng 125100, Liaoning, China; ²Weixian Forestry-Fruit Development Service Center, Weixian 056800, Hebei, China)

Abstract: 【Objective】 Weixian has a long pear cultivation history with approximately 20 unique local pear cultivars, including the most famous Weixian Yali. Researchers have made significant progress in the collection, preservation and phenotypic evaluation on pear germplasm resources in Weixian, but the genetic relationship and background of these pears remain largely unexplored. To provide a theory basis for the efficient utilization of Weixian pears and the sustainable development of pear industry, it is needed to elucidate the genetic relationship and background of Weixian pear accessions. 【Methods】 Twenty local pear cultivars from Weixian and 10 representative local pear cultivars from other regions within Hebei province were selected as research materials. Based on the whole-genome resequencing data and chloroplast DNA variation, we investigated the genetic relationship and background of local pear cultivars in Weixian. The clean reads were aligned to the reference genome Yunhong No. 1 using BWA v0.7.15 software for whole-genome alignment; SNP detection and filtering were performed using

收稿日期: 2024-12-25 接受日期: 2025-03-02

基金项目: 中国农业科学院科技创新工程(CAAS-ASTIP-2021-RIP-01); 财政部和农业农村部: 国家现代农业产业技术体系(CARS-28-01)

作者简介: 齐丹, 女, 硕士, 研究方向为梨种质资源。E-mail: qidan@caas.cn

*通信作者 Author for correspondence. E-mail: dongxingguang@caas.cn; E-mail: hdwxljzj@163.com

GATK v4.2.5.0 software; Population structure analysis was conducted using Admixture v1.3.0 and principal component analysis was carried out using GCTA v1.93.2 software; Treebest v1.9.2 software was used to calculate the distance matrix, and a phylogenetic tree was constructed using the neighbor-joining method. The chloroplast DNA sequencing results were aligned and manually corrected using the software MEGA X; the genetic parameters of chloroplast DNA sequence fragments were calculated using the software DnaSP 5.1; the evolutionary relationship between haplotypes was analyzed using the software NetWork 10. **【Results】** By comparing the resequencing data of 30 materials with the reference genome, a total of 30 041, 877 SNP loci were identified. We performed principal component analysis, population genetic structure and phylogenetic tree based on these SNPs. The results showed that local pear cultivars in Weixian exhibited a clear population genetic structure, which could be divided into two groups: Group 1 consisted of 9 materials including Weixian Hongli, Youqiu, Xiaohongmian, Baidunzili, Baihongli, Duzili, Jinfeng Yali, Kaixinmian and Suanjiuli; Group 2 comprised the remaining 11 materials from Weixian, including Xiaobaimian, Xuehua, Wu'an Youqiu, Dabaimian, Yayamian, Teda Baimian, Baixuehua, Yinbai, Yali, Teda Yali, Meixiang Yali and Xiaobaimian, which can be further divided into two subgroups. Additionally, Group 2 included 10 local pear cultivars from northern Hebei, like Anli and Baihuaguan, indicating a close genetic relationship between Weixian pears in Group 2 and northern pear cultivars. In contrast, Weixian Hongli in Group 1 belonged to the Chinese Sand Pears, suggesting a closer genetic relationship between Weixian pears in Group 1 and southern pear cultivars. The sequencing results of the non-coding region of chloroplast DNA showed that three more evolved haplotypes were detected in Weixian pears. The haplotype of most Weixian pears was haplotype H_3, while Weixian Hongli exhibited the haplotype H_2. Additionally, Xuehua and the Yali group, with the exception of Jinfeng Yali, displayed the haplotype H_1. By analyzing the genetic relationship and haplotype categories, we identified the genetic background of bud sport mutations and hybrids of Weixian pears. Jinfeng Yali belonged to the Yali group and had the characteristic of high self-fertility. Whole-genome resequencing analysis and chloroplast haplotype results showed that Yali, Meixiang Yali and Teda Yali belonged to the same branch and had the same haplotype. The clustering results and haplotype of Jinfeng Yali were different from these three Yali pear varieties. Jinfeng Yali was more closely related to Suanjiuli, and both had the same chloroplast haplotype, indicating that Jinfeng Yali was not a bud sport of Yali but possibly a selection from Yali seedlings. Duzili and Baidunzili had a close genetic relationship and shared the same haplotype, suggesting Baidunzili may be a bud sport of Duzili. The chloroplast haplotypes of Baihongli and Weixian Hongli were inconsistent, but they had a close relationship, suggesting that Weixian Hongli was more likely to be its paternal variety. Kaixinmian and Xiaohongmian shared the same chloroplast haplotype, and Xiaohongmian and Duzili were in the same group, suggesting that Kaixinmian was a hybrid between Xiaohongmian and Duzili. There was a distinct genetic difference between Youqiu and Wu'an Youqiu, despite their similar names. **【Conclusion】** In this study, based on the information of single nucleotide polymorphisms (SNPs) revealed by whole-genome resequencing and chloroplast DNA variation, we clarified the genetic relationship and background of Weixian pears. This result provides valuable references for protection and rational utilization of Weixian pear germplasm resources, further aiding in advancing the genetic understanding of pear cultivation in the region. Additionally, it enriches the cultural significance of pear culture in Weixian and offers cultural support for rural revitalization efforts.

Key words: Weixian pear; Whole-genome resequencing; Chloroplast DNA; Genetic relationship

魏县地处河北省南部,位于北纬36°黄金种植带上,气候温和且四季分明,光热资源丰富,是古黄河及漳卫河冲积而成的山前平原,土质肥沃,为梨树的最适宜栽培区。魏县梨树栽培历史悠久,地方品种资源丰富,约有20种,其中最为知名的为魏县鸭梨,其他品种作为鸭梨授粉品种小面积栽培。2007年鸭梨被批准为国家地理标志保护产品,栽培面积超过1万hm²。魏县鸭梨栽培具有2000多年的历史,据考证,东汉末年孔融让梨的典故中所让之梨即为魏县鸭梨,而南北朝著名科学家贾思勰所著《齐民要术》中对魏县鸭梨种植情况也作了详细描述。因此,梨树栽培在魏县不仅是重要的经济收入来源,更成为一种文化符号在魏县世代相传。但近年来随着人民生活水平不断提高,消费者对果实品质的要求也越来越高,同时我国果树育种进程持续加快,新品种不断涌现,因此梨果市场竞争激烈,如何能够实现鸭梨品种改良及品质提升从而保持魏县鸭梨的品牌效应,是产业亟待解决的主要问题。魏县鸭梨在多年的栽培过程中,发现并积累了多个自发的鸭梨新变异材料,其中包括果点少的美香鸭梨^[1],其果点只有鸭梨的13.4%,以及自交亲和的金丰鸭梨,其自花结实率达到50%。除鸭梨外,魏县古老地方品种有魏县红梨、银白、紫酥梨、砣子梨、油秋、白雪花、小红面、小白面、大白面、鸭鸭面等。其中魏县红梨是魏县独有的古老地方品种,其种植历史悠久,至迟应始于三国魏黄初年间(220—226年),红梨果功效奇特,既可鲜食,又可加工,医疗保健作用明显,魏县红梨是鸭梨最好的授粉树^[2]。在生产中这些地方品种又衍生出一批优秀的地方品种资源,例如:疑似银白和魏县红梨杂交实生获得的白红梨,小红面为母本、砣子梨为父本杂交获得的开心面;疑似砣子梨芽变选育的白砣子等。阐明鸭梨优系以及地方品种的遗传背景和亲缘关系,对实现魏县梨品种资源的高效利用以及梨产业可持续发展具有重要的指导意义。

DNA分子标记常用来鉴定果树种质资源的遗传多样性和亲缘关系,其中简单重复序列(simple sequence repeats, SSR)在梨属植物的遗传研究上应用较多^[3-6]。尽管SSR标记在梨属植物上的位点数量较少,但随着高通量测序技术的发展,梨属植物的SSR位点数量得以提升^[7-8]。同SSR标记相比,单核苷酸多态性(single nucleotide polymorphism, SNP)

作为第三代分子标记,具有数量极多、多态性丰富、具二态性、可实现高通量自动化检测的特点^[9]。目前多个梨属植物基因组的测序与组装已完成^[10-14],在此基础上,利用全基因组重测序(whole genome re-sequencing, WGS)技术对不同梨个体的基因组进行测序,与参考基因组进行比对,利用生物信息学手段,可以揭示大量单核苷酸多态性(SNP)、插入/缺失(InDel)以及结构变异(SVs)等遗传变异信息,开展梨属植物演化、遗传变异等研究^[15-17]。梨属植物的叶绿体DNA具有母系遗传的特性,利用其非编码区域的基因间区存在丰富的遗传变异,开展了梨属植物的分类^[18]、起源演化^[19]和遗传多样性评价^[20]等研究。将双亲遗传的核基因标记和母系遗传的叶绿体变异信息结合起来,可更全面地解析梨种质资源的起源演化和亲缘关系^[21]。

魏县主管生产单位十分重视梨地方品种的保护,21世纪以来成立“鸭梨变异品系筛选试验”、“红梨选优及配套栽培技术研究”等课题组,历经10余年优系筛选工作,建立鸭梨优系及魏县原产梨地方品种的完整档案并实现资源的妥善保存,这些宝贵的品种资源为魏县鸭梨及地方品种的改良提供了重要的基因基础。笔者在本研究中以河北魏县鸭梨变异材料以及地方梨品种为主要研究对象,基于全基因组重测序的单核苷酸多样性及叶绿体DNA序列变异信息,对魏县梨品种资源进行叶绿体单倍型、群体遗传结构、系统进化等分析,解析河北魏县梨地方品种遗传背景,探讨梨种质间的亲缘关系,为魏县梨品种优化改良奠定理论基础,同时推进魏县梨文化基因解码,丰富其文化内涵,助力文化融合和三产协调发展,为乡村振兴提供文化支撑。

1 材料和方法

1.1 试验材料

采集20个魏县梨地方品种的幼叶,由魏县林果开发服务中心提供;从国家梨、苹果种质资源圃(辽宁兴城)采集起源于河北其他地区代表性梨地方品种10个,共计30个河北地方品种为研究材料,材料具体信息见表1。

1.2 基因组DNA提取

采用改良的CTAB法提取梨嫩叶DNA,通过0.8%琼脂糖凝胶电泳和微量分光光度计Nano-200(奥盛仪器有限公司,杭州)检测DNA质量和浓度。

表 1 材料信息

Table 1 Plant materials used in this study

编号 Code	种质名称 Accession name	种类 Species	来源地 Origin	果实性状 Fruit characters
1	美香鸭梨 Meixiang Yali	白梨 <i>P. bretschneideri</i>	河北魏县 Weixian, Hebei	果实中大,平均单果质量220 g,果点稀少(是鸭梨果点数的13.4%),果肉乳白色,香味浓郁,贮藏后,香味更浓,甜酸适口,成熟期比鸭梨晚7 d左右,可溶性固形物含量最高可达14.5%。其他性状与鸭梨相同。 Fruit size medium to large, average single fruit weight 220g, fruit dot rare (only 13.4% of the quantity in Yali), flesh creamy white, sweet-sour, aromatic, aromatic stronger after storage, maturation period 7 days later than Yali, soluble solids content 14.5% at peak level, other characteristics align with those of Yali.
2	魏县红梨 Weixian Hongli	砂梨 <i>P. pyrifolia</i>	河北魏县 Weixian, Hebei	果实倒卵圆形,单果质量102~161 g;果皮厚而坚韧,赤褐色,果面平滑,有蜡质;果点多,圆小,褐色,稍突出;果肉淡黄色,肉质致密,石细胞中多,果汁多,味酸甜,有微香,可溶性固形物含量11.2%,滴定酸含量0.583%,品质上等。 Fruit shape obovate, single fruit weight 102-161g, thick and leathery russet-colored skin, smooth with a waxy coating, fruit dots abundant and small, brown, slightly raised, flesh pale yellow, dense texture, moderate stone cell content, juicy, sour-sweet, subtle fragrance, soluble solids content 11.2%, titratable acidity 0.583%, quality good.
3	特大鸭梨 Teda Yali	白梨 <i>P. bretschneideri</i>	河北魏县 Weixian, Hebei	果实特大,平均单果质量516 g,果点中等而密,果梗长,鸭突明显,萼片脱落。果肉白色,酥脆多汁、香味浓。果皮薄,成熟时绿黄色,套袋果黄白色。果实9月中旬成熟,比鸭梨早成熟5~7 d。耐贮藏,常温可存2个月,冷藏可贮至翌年5月份。 Fruit size exceptionally large, average single fruit weight 561g, fruit dots moderate, densely distributed, long stalk, prominent calyx end protrusion, deciduous sepals, flesh white, crisp, juicy, aromatic, thin skin, greenish-yellow, or yellowish-white when bagged, fruit ripens in mid-September, 5-7 days earlier than Yali, good storage tolerance, maintaining quality for up to 2 months at room temperature, remaining fresh under refrigeration until May of the following year.
4	金丰鸭梨 Jinfeng Yali	白梨 <i>P. bretschneideri</i>	河北魏县 Weixian, Hebei	果实卵圆形,平均单果质量228 g,果肉白色,果梗长,基部肉质、鸭突明显,酸甜适口,香气浓郁,成熟期9月中下旬,可溶性固形物含量11%,较耐贮藏,品质上。其他性状同鸭梨。 Fruit shape ovate, average single fruit weight 228g, flesh white, long stalk, fleshy base, prominent calyx end protrusion, sour-sweet, distinct aromatic, ripening in late September, soluble solids content 11%, moderate storage tolerance, quality good, other characteristics align with those of Yali.
5	银白 Yinbai	白梨 <i>P. bretschneideri</i>	河北魏县 Weixian, Hebei	果实倒卵圆形或近圆形,单果质量126~234 g;果皮淡绿黄色,光滑,稍坚韧;果点多,圆形,锈褐色;果肉白色,肉质细而脆,石细胞少,果汁多,味极甜,略有芳香,可溶性固形物含量13%以上,品质上等。 Fruit shape obovoid or sub-round, single fruit weight 126-234 g, pale greenish-yellow skin, smooth, slightly firm, fruit dots numerous small, round and rust-brown, flesh white, fine-textured and crisp, minimal stone cells, juicy, strongly sweet, subtle aromatic, soluble solids content above 13%, quality good.
6	白红梨 Baihongli	白梨 <i>P. bretschneideri</i>	河北魏县 Weixian, Hebei	果实倒卵圆形或椭圆形,单果质量83~138 g;果皮棕黄色,平滑,厚而韧;果点多,中等大,圆形,锈褐色;果肉黄白色,质硬脆,石细胞多,果汁中多,味甜酸,微有香气,品质中等。果实耐运输和贮藏。 Fruit shape obovoid or elliptic, single fruit weight 83-138 g, brownish-yellow skin, smooth, thick and tough, fruit dots numerous, medium-sized, round, rust-brown, flesh yellowish-white, firm and crisp, abundant stone cells, mid-juicy, sweet-sour, subtle aromatic, medium quality, good tolerance for transportation, storage life long.
7	砣子梨 Dunzili	白梨 <i>P. bretschneideri</i>	河北魏县 Weixian, Hebei	果实近圆形,单果质量111~205 g;果面锈褐色,稍平滑,果皮较厚而坚韧;果点多,圆形,淡褐色;果肉水白色,肉质致密而脆,石细胞中多,果汁多,味甜,微具微香,可溶性固形物含量9.86%,可滴定酸含量0.258 6%,品质上等。果实极耐贮藏和运输,贮藏期180 d以上。 Fruit shape sub-round, single fruit weight 11-205 g, russet-brown skin, slightly smooth, thick and tough, fruit dots numerous, pale brown, small and round, flesh creamy-white, dense and crisp, medium stone cell, juicy, sweet, subtle aromatic, soluble solids content 9.86%, titratable acidity 0.258 6%, quality good, excellent storage and transport tolerance, storage period exceeding 180 days.
8	白砣子 Baidunzi	白梨 <i>P. bretschneideri</i>	河北魏县 Weixian, Hebei	果实椭圆形,中大,一般单果质量70~100 g,果皮黄绿色,有蜡质光泽,果点小而密,果实外形美观。果肉白色,质脆,多汁,甜味浓,品质一般。 Fruit shape elliptic, medium-sized, single fruit weight 70-100 g, yellow-green skin, waxy sheen, fruit dots small and densely, appealing appearance, flesh white, crisp, juicy, intensely sweet, medium quality.
9	大白面 Dabai-mian	白梨 <i>P. bretschneideri</i>	河北魏县 Weixian, Hebei	果实近圆形或长卵圆形,单果质量104~178 g;果皮厚而坚韧,稍粗糙,黄绿色;果点圆形,锈色,较显著;果肉白色,后熟后肉质变绵软,石细胞较少,果汁中多,味甜酸,略有芳香,品质中等。后熟期长,一般需要100 d左右,故也叫“百天面”。果实可贮藏150 d。 Fruit shape sub-round or long ovate, single fruit weight 104-178 g, yellowish-green skin, thick and tough, slightly rough, fruit dots prominent, rust-colored and round, flesh white, crisp-soft, minimal stone cells, mid-juicy, sweet-sour, subtle aromatic, medium quality, long post-ripening period, approximately 100 days, storage life long, 150 days.

表1 (续)
Table 1 (Continued)

编号 Code	种质名称 Accession name	种类 Species	来源地 Origin	果实性状 Fruit characters
10	小白面 Xiao- baimian	白梨 <i>P.</i> <i>bretschneideri</i>	河北魏县 Weixian, Hebei	果实较小,圆形,单果质量38~51 g;果皮厚而坚韧,黄色,果面平滑,略有蜡质;果点小,圆形,锈色,稍突出;果肉乳黄色,石细胞多,后熟后肉质变松软,味甜,品质中等,后熟期30 d左右。果实不耐贮藏。 Fruit shape round, small size, single fruit weight 38–51 g, yellow skin, thick and tough, smooth, a slight waxy coatin, fruit dots small, round and rust-colored, slightly raised, flesh creamy-yellow, abundant stone cells, soft- tender texture after post-ripening, sweet, medium quality, post-ripening period about 30 days, storage life short.
11	鸭鸭面 Yayamian	白梨 <i>P.</i> <i>bretschneideri</i>	河北魏县 Weixian, Hebei	果实圆形或扁圆形,单果质量65~93 g;果皮厚而坚韧,平滑,黄绿色,后熟后变黄色;果点密而小,圆形,褐色;果肉白色,后熟后肉质变绵软,石细胞中多,果汁中多,味甜,品质中等,后熟期20 d左右。果实较耐运输,可贮藏100多天。 Fruit shape round or oblate, single fruit weight 65–93 g, yellowish-green skin, yellow after post-ripening, thick and tough, smooth, fruit dots dense, small, round and brown, flesh white, turning to melting after post-ripening, moderate stone cell content, mid-juicy, sweet, medium quality, post-ripening period approximately 20 days, good transport tolerance, storage life over 100 days.
12	小红面 Xiao- hongmian	白梨 <i>P.</i> <i>bretschneideri</i>	河北魏县 Weixian, Hebei	果实扁圆形,甚小,单果质量24~36 g;果面全部赤褐色,稍平滑,有蜡质,果皮厚而坚韧;果点多而明显,圆形,褐色;果肉白色,石细胞中多,有涩味,经后熟后肉质变松软,果汁中多,味酸,微具微香,品质中等。果实后熟10余天,不耐贮藏。 Fruit shape oblate,exceptionally small size, single fruit weight 24–36 g, russet-brown skin, thick and tough,slightly smooth, waxy coating, fruit dots numerous prominent, round and brown, flesh white, moderate stone cell content, slightly astringent, flesh turning to soft after post-ripening, mid-juicy, sour, subtle aromatic, medium quality, post-ripening period around 10 days, storage life short.
13	特大白面 Teda Baimian	白梨 <i>P.</i> <i>bretschneideri</i>	河北魏县 Weixian, Hebei	果实特大型,平均单果质量360 g,果近似椭圆形,果点中大、中密,果柄较短,萼洼深,萼宿存。果肉白色稍粗,汁中多,果实9月中下旬成熟,采摘时口感硬脆,甜稍酸,自然存放2个月,果实变面变松。 Fruit shape approximately elliptic, exceptionally large-sized, single fruit weight 360 g, fruit dots medium-sized, moderately dense, short stalk, deep calyx basin, persistent calyx, flesh white, slightly coarse, mid-juicy, ripening in mid-to-late September, firm-crisp, sweet-sour, flesh softens to a mealy texture after two months of natural storage.
14	油秋 Youqiu	白梨 <i>P.</i> <i>bretschneideri</i>	河北魏县 Weixian, Hebei	果实圆形,单果质量81~107 g;果皮较厚,坚韧,黄绿色,果面略平滑;果点多,圆形,锈褐色;果肉白色,致密而脆,果汁中多,味酸甜,有香气,品质中等。果实耐贮藏和运输,贮藏后品质有所提高,贮藏期230多天。 Fruit shape globose, single fruit weight 81– 107 g, yellowish- green skin, thick and tough, slightly smooth, fruit dots numerous small, round and rust- brown, flesh white, dense and crisp, mid-juicy, sour-swee, distinct aromatic, medium quality, excellent tolerance for storage and transportation, quality improved after storage, storage period exceeds 230 days.
15	武安油秋 Wu'an Youqiu	白梨 <i>P.</i> <i>bretschneideri</i>	河北魏县 Weixian, Hebei	果实卵圆形,单果质量167.3 g,果皮绿黄色,阳面无红晕;果心小,5心室;果肉白色,肉质细脆,汁液多,味甜酸,无香味;可溶性固形物含量11.0%;品质中等,常温下可贮藏90 d。 Fruit shape ovate, average single fruit weight 167.3 g, greenish-yellow skin, no reddish blush on sun side, small core, 5 carpels, flesh white, fine and crisp, juicy, sweet-sour, no distinct aromatic, soluble solids content 11.0%, medium quality, stored for 90 days under room temperature.
16	蒜白 Suanjiu	白梨 <i>P.</i> <i>bretschneideri</i>	河北魏县 Weixian, Hebei	果实大,椭圆形或长卵圆形,单果质量305~578 g;果皮较厚而坚韧,果面粗糙,黄绿色;果点特大,圆形,锈色,分布均匀;果肉白色,肉质致密而稍细,石细胞较少,果汁多,味甜酸,无香气,品质上等。果实耐贮藏和运输。一般可贮藏200多天,经贮藏后风味更佳。 Fruit shape elliptic or long ovate, large-sized, single fruit weigh 305–578 g, yellowish- green skin, thick and tough, rough surface, fruit dots exceptionally large, round and rust-colored, evenly distributed, flesh white, dense and slightly fine, minimal stone cells, juicy, sweet-sour, no distinct aromatic, quality good, excellent tolerance for transportation and storage, storage period exceeding 200 days, flavor improved significantly after storage.
17	鸭梨 Yali	白梨 <i>P.</i> <i>bretschneideri</i>	河北魏县 Weixian, Hebei	果实中大,一般单果质量200~240 g。果实倒卵圆形,果肩一侧鸭头状突起。果梗细长,常弯向一侧,基部肉质化,脱萼,梗洼深广。采收时果面绿黄色,贮后黄白色,果皮薄,果梗附近有锈斑,果点小而密,果实美观;果心小、果肉白色、肉质特细而脆嫩,汁多味甜,可溶性固形物含量11%~13%,有香气,石细胞极少,品质上。果实9月中下旬成熟,较耐贮藏,可贮藏至翌年2—3月份。 Fruit shape obovate, medium- large sized, single fruit weight 200–240 g, a duck-head-shaped protrusion on one shoulder, slender and often curved stalk with a fleshy base, deciduous sepals, broad and deep stalk cavity, greenish- yellow skin at harvest, turning pale yellow after storage, rust-colored patches near the stalk base, fruit dots small and dense, appealing appearance, flesh white, small core, an exceptionally fine and crisp, juicy, sweet, soluble solids content 11%–13%, aromatic, minimal stone cells, superior quality, mid-to-late September ripening good storage tolerance, remaining fresh until February-March of the following year.

表 1 (续)
Table 1 (Continued)

编号 Code	种质名称 Accession name	种类 Species	来源地 Origin	果实性状 Fruit characters
18	白雪花 Baixue- hua	白梨 <i>P.</i> <i>bretschneideri</i>	河北魏县 Weixian, Hebei	果实宽倒卵圆形或近圆形,单果质量137~244 g;果面黄色,光滑,皮稍厚;果点较大,呈不规则形,锈色,密布于果面;果肉白色,肉质稍细,脆而致密,石细胞较少,果汁多,味很甜,可溶性固形物含量达14%,品质上等。耐贮性好。 Fruit shape broadly obovate or sub-round, single fruit weight 137-244 g, yellow skin, smooth, slightly thick, fruit dots large, irregularly shaped, rust-colored and dense, flesh white, slightly fine, crisp-dense, few stone cells, juicy, intensely sweet, soluble solids content 14%, superior quality, excellent storage tolerance.
19	开心面 Kaixin- mian	白梨 <i>P.</i> <i>bretschneideri</i>	河北魏县 Weixian, Hebei	果面暗褐色,略粗糙,果皮厚而坚韧;果点多而大,圆形,浅褐色;果梗褐色,长2.5~3.0 cm;梗洼深,中广;萼洼深广,萼残存;后熟需约10 d,果肉黄白色,肉质绵,味甜,可溶性固形物含量16%以上,品质上等。 Dull brown skin, slightly rough surface, thick-tough, fruit dots numerous large, round, light brown, brown stalk measures 2.5-3.0 cm in length, with a deep and moderately broad stalk cavity, deep and wide calyx basin, persistent calyx, post-ripening period approximately 10 days, flesh creamy-yellow, soft, mealy texture, sweet, soluble solids content exceeding 16%, superior quality.
20	雪花 Xuehua	白梨 <i>P.</i> <i>bretschneideri</i>	河北魏县 Weixian, Hebei	果实长卵圆形或长圆形。果实大型,一般单果质量250~300 g。梗洼浅,有少量锈斑,萼片脱落,萼洼深广。果皮厚、绿黄色、果面稍粗,果点小,贮后果皮金黄色,具蜡质光泽,外形美观。果心较小,果肉白色,质硬脆、稍粗、汁液中度、味甜,可溶性固形物含量12%,微香,品质上。果实9月上旬成熟,耐贮运。 Fruit shape elongated ovoid or oblong, large-sized, single fruit weight 250-300 g, shallow stalk cavity with minor rust-colored patches, deciduous sepals, deep and broad calyx basin, greenish-yellow skin, thick, slightly rough, small fruit dots, after storage skin turning golden yellow with a waxy sheen, giving the fruit an attractive appearance, flesh white, firm and crisp, slight coarse, mid-juicy, sweet, soluble solids content 12%, subtle aromatic, superior quality.
21	油瓶 Youping	白梨 <i>P.</i> <i>bretschneideri</i>	河北石家庄 Shijiahuang, Hebei	果实纺锤形/葫芦形,平均单果质量217 g,内质综合评价中,果肉质地稍脆;果肉较细;汁液中多。 Fruit shape spindle-shaped or pear-formed, average single fruit weight 217 g, quality medium, flesh slight crisp, relatively-fine, mid-juicy.
22	大紫酥 Dazisu	白梨 <i>P.</i> <i>bretschneideri</i>	河北大名 Daming, Hebei	果实类型脆肉型,风味酸甜,果实近圆形,平均单果质量203 g,内质综合评价中,果肉质地紧脆,果肉中粗,汁液中多。 Flesh texture type crisp, sour-sweet, fruit shape sub-round, average single fruit weight 203 g, quality medium, flesh dense and crisp, mid-coarse, mid-juicy.
23	安梨 Anli	秋子梨 <i>P.</i> <i>ussuriensis</i>	河北、东北 Hebei, Northeast of China	果实类型软肉型,风味/香气酸稍涩/微香,果实形状扁圆形,平均单果质量104 g,内质综合评价中,果肉质地紧密一软,果肉粗,汁液多。 Flesh texture type soft, slight astringency/faintly aromatic, fruit shape oblate, mass of single fruit: 104 g; quality evaluation: medium; flesh dense and crisp, coarse, turning to soft after ripening, juicy.
24	胎黄 Taihuang	白梨 <i>P.</i> <i>bretschneideri</i>	河北交河 Jiaohe, Hebei	果实类型脆肉型,风味甜,果实长圆形,平均单果质量87 g,内质综合评价中,果肉质地松脆,果肉中粗,汁液中多。 Flesh texture type: crisp; flavor: sweet; fruit shape: long globose, average single fruit weight 87 g, quality medium, flesh crisp tender, mid-coarse, mid-juicy.
25	金酥糖 Jinsutang	白梨 <i>P.</i> <i>bretschneideri</i>	河北青龙 Qinglong, Hebei	果实类型脆肉型,风味淡甜,果实圆形,平均单果质量80 g,内质综合评价中,果肉质地松脆,果肉中粗,汁液中多。 Flesh texture type crisp, light sweet, fruit shape globose, average single fruit weight 80 g, quality medium, flesh crisp tender, mid-coarse, mid-juicy.
26	红枝母秧 Hongzhi Muyang	白梨 <i>P.</i> <i>bretschneideri</i>	河北兴隆 Xinglong, Hebei	果实类型脆肉型,风味酸甜,果实圆形,平均单果质量122 g,内质综合评价中上,果肉质地脆,果肉较细,汁液中多。 Flesh texture type crisp, sour-sweet, fruit shape globose, average single fruit weight 122 g, quality above medium, flesh crisp tender, relatively-fine, mid-juicy.
27	白枝母秧 Baizhi Muyang	白梨 <i>P.</i> <i>bretschneideri</i>	河北兴隆 Xinglong, Hebei	果实类型脆肉型,风味淡甜,果实扁圆形,平均单果质量107 g,内质综合评价中上,果肉质地脆,果肉较细,汁液中多。 Flesh texture type crisp, light sweet, fruit shape oblate, average single fruit weight 107 g, quality above medium, flesh crisp, relatively-fine, mid-juicy.
28	佛见喜 Fojianxi	白梨 <i>P.</i> <i>bretschneideri</i>	河北遵化 Zunhua, Hebei	果实类型脆肉型,风味甜,果实扁圆形,平均单果质量144 g,内质综合评价上,果肉质地松脆,果肉粗细较细,汁液多。 Flesh texture type crisp, sweet, fruit shape oblate, average single fruit weight 144 g, quality good, flesh crisp tender, relatively-fine, juicy.
29	鸡蛋罐 Jidan- guang	白梨 <i>P.</i> <i>bretschneideri</i>	河北青龙 Qinglong, Hebei	果实类型脆肉型,风味酸甜,果实卵圆形,平均单果质量110 g,内质综合评价中上,果肉质地松脆,果肉较细,汁液中多。 Flesh texture type crisp, sour-sweet; fruit shape ovate, average single fruit weight 110 g, quality above medium, flesh crisp tender, relatively-fine, mid-juicy.
30	白花罐 Baihua- guang	秋子梨 <i>P. ussuriensis</i>	河北抚宁 Funing, Hebei	果实类型软肉型,风味/香气甜酸/微香,果实圆形,平均单果质量124 g,内质综合评价中下,果肉质地紧密一软,果肉粗,汁液中多。 Flesh texture type soft, sour-sweet, slightly aromatic, fruit shape globose, average single fruit weight 124 g, quality lower medium, flesh dense-soft, coarse, mid-juicy.

1.3 叶绿体非编码区序列扩增、测序及数据分析

选择5对叶绿体通用引物对叶绿体非编码区进行扩增,引物序列和扩增程序参照Chang等^[19]的报道,对PCR产物进行正反双向测序,由美吉生物(北京)有限公司完成。利用软件MEGA X软件^[22]对测序结果进行序列比对和人工校正;使用软件DnaSP 5.1^[23]计算叶绿体DNA序列片段的遗传参数;利用软件NetWork 10^[24]分析单倍型之间的进化关系。

1.4 重测序文库构建和数据分析

使用高通量测序仪Novaseq产生原始测序序列(Raw Reads),结果以FASTQ文件格式存储,去除里面含有带接头的、重复的、测序质量低的序列,得到高质量测序数据(clean read)后,采用BWA v0.7.15软件^[25]将高质量测序数据与参考基因组云红1号(http://pearomics.njau.edu.cn/sites/default/files/raw/Pyrus_pyrifolia_Yunhong_NO.1_v1.0/YunhongNO.1_genome.fasta)做全基因组比对,初始比对结果为sam格式,再利用SAMtools v1.4软件^[26],将结果转为bam格式并排序,然后进行数据基本信息统计及比对统计。基于测序产生序列与云红1号参考基因组序列的比对结果,采用GATK v4.2.5.0软件^[27]对单核苷酸多态性位点进行检测和过滤,然后使用Admixture v1.3.0^[28]进行群体结构分析,使用GCTA v1.93.2软件^[29]进行主成分分析,使用Treebest v1.9.2软件(<https://treesoft.sourceforge.net/treebest.shtml>)计算距离矩阵,通过邻接法(neighbor-joining method)构建系统发育树。

2 结果与分析

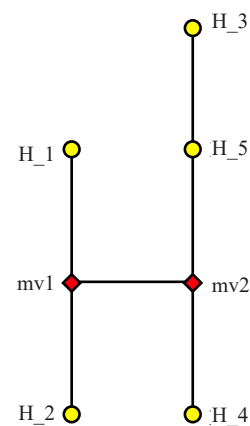
2.1 叶绿体非编码区序列多态性和品种单倍型信息

将叶绿体通用引物P02、P03、CP03、CP11和P09扩增到的到序列进行比对校正和片段合并,得到的序列长度为4588 bp,包含多态性位点11个,其中单一突变位点2个,简约信息性位点3个,插入/缺失片段6个。合并之后的叶绿体基因片段,核苷酸多样性(Pi)、平均核苷酸差异(K)和单倍型多样性(Hd)分别为0.000 46、1.570 11和0.566。在30个梨属品种中检测到5个单倍型,H₁、H₂、H₃、H₄和H₅,数量分别为9个、1个、18个、1个和1个。20个魏县梨地方品种中检测到3种单倍型,其中鸭梨、特大

鸭梨、美香鸭梨和雪花的单倍型是H₁,魏县红梨的单倍型是H₂,其他15个品种的单倍型是H₃。10个来自河北其他地区的梨地方品种中检测到4种单倍型,油瓶、胎黄、红枝母秧、白枝母秧和佛见喜的单倍型为H₁,大紫酥、金酥棠和鸡蛋罐的单倍型为H₃,安梨的单倍型为H₄,白花罐的单倍型为H₅。

2.2 基于5个叶绿体基因片段合并数据的单倍型间的中介网络图的构建

合并之后的叶绿体基因片段,Tajima's D值为0.665 81, $p>0.10$ 时不显著,目标序列在进化上遵循中性模型,序列可用于系统发育分析。运用中介邻接网络(Median-Joining network, MJ)算法,并用最大简约(Maximum Parsimony calculation, MP)算法来优化由MJ计算过的结果,构建中国河北地区梨属种质叶绿体DNA单倍型间的关联图(图1)。单倍型



H₁. 单倍型 1; H₂. 单倍型 2; H₃. 单倍型 3; H₄. 单倍型 4; H₅. 单倍型 5; mv1. 中介矢量载体 1; mv2. 中介矢量载体 2。

H₁. Haplotype1; H₂. Haplotype2; H₃. Haplotype3; H₄. Haplotype4; H₅. Haplotype5; mv1. Medium vector1; mv2. Medium vector2.

图1 单倍型H₁~H₅的中介邻接网络

Fig. 1 Median-joining network of haplotypes H₁-H₅

和中介矢量载体的名称分别表示为H和mv。结果显示5个单倍型均不在中介网络图的躯干(torso)上,说明5个单倍型的分化时间晚,是比较进化的单倍型。H₁和H₂由mv1连接,存在1个碱基变异和2个InDel(插入/缺失),H₄和H₅由mv2连接,两者之间有2个碱基变异和1个InDel,H₃从H₅延伸出来,两者亲缘关系较近,存在1个碱基差异和1个InDel。不同单倍型之间的差异见表2。

2.3 全基因组重测序和主成分分析

在得到Clean Reads后,将Clean Reads与参考基

表 2 5个单倍型的叶绿体序列变异位点

Table 2 The sequence variations among 5 haplotypes

变异位点 Variation site	单倍型 1 Haplotype 1	单倍型 2 Haplotype 2	单倍型 3 Haplotype 3	单倍型 4 Haplotype 4	单倍型 5 Haplotype 5
BC1	G	G	C	G	G
InDel1			+22 bp		
InDel 2			+229 bp	+229 bp	+229 bp
InDel3	+1 bp				
BC2	T	T	C	C	C
BC3	T	G	T	T	T
InDel 4				+8 bp	
InDel 5	+10 bp				
BC4	T	T	A	T	A
BC5	T	T	T	G	T

注:BC 为点突变;InDel 代表插入/缺失片段。

Note: BC stands for Base Change, which refers to point mutations; InDel represents insertions/deletions.

基因组作比对,初始比对结果为 sam 格式,再利用 SAMtools 软件将结果转为 bam 格式并排序,并去除重复序列,然后进行数据基本信息统计及 map 比对统计,统计结果如下:30 份样品过滤后 reads 数为 352 811 60~764 130 40,比对 reads 数为 346 587 50~750 367 79,比率为 97.52%~98.44%,平均测序深度为 9.05~19.45 X,覆盖率为 94.15%~97.81%,详情见表 3。共得到高质量的 SNPs 300 418 77 个,用于下游分析。

使用 GCTA 软件进行主成分分析(PCA),第一主成分和第二主成分分别解释了总遗传变异的 27.96%和 14.83%。在主坐标 1 方向上可将 30 个地方品种清晰地分为两个群组,其中群组 1 由雪花、武安油秋、大白面、鸭鸭面、银白、特大白面、白雪花、小白面、特大鸭梨、鸭梨、美香鸭梨 11 个魏县地方品种和 10 个河北其他地区的品种,群组 2 由魏县红梨、小红面、白砣子、油秋、蒜白、砣子梨、白红梨、开心面、金丰鸭梨共 9 个魏县地方品种组成;在主坐标轴 2 个方向上可将群组 1 的 21 个地方品种分为 2 个亚组,亚组 1 由小白面、特大鸭梨、鸭梨、美香鸭梨 4 个魏县地方品种和 10 个河北其他地区的品种组成,亚组 2 由雪花、武安油秋、大白面、鸭鸭面、银白、特大白面、白雪花 7 个魏县地方品种组成(图 2-A)。

2.4 系统进化分析

基于群体中每个样品的 SNP 信息,使用 Treebest(v1.9.2)软件计算距离矩阵,通过邻接法(neighbor-joining method)构建系统发育树。进化树将 30

个品种可分为 群组 1 和 群组 2,不同群组之间差异显著,而同一群组的品种亲缘关系比较近。品种组成与主成分分析的结果一致;群组 2 可划分为两个亚群,亚群 1 由魏县梨地方品种小白面和 9 个河北其他地区的梨地方品种组成,亚群 2 主要由魏县梨地方品种组成,包括鸭梨、美香鸭梨等,还包括 1 个来自河北其他地区的油瓶,除油瓶和小白面外,2 个亚组的品种组成与主成分分析结果一致(图 2-B)。

2.5 群体遗传结构分析结果

使用 Admixture 软件进行群体遗传结构分析,不同 K 值下的种群结构分析结果如下:当 K 值设定为 2 时,魏县红梨等 9 个魏县梨地方品种构成类群 1,其他品种组成类群 2;当 K 值增加到 3 时,类群 1 仍是魏县红梨等 9 个魏县梨地方品种,原来类群 2 的品种被进一步细分,由鸭梨等 4 个魏县地方品种和 10 个河北其他地区的品种组成类群 2,雪花等 7 个魏县梨地方品种组成类群 3;当 K 为 4 时,鸭梨、美香鸭梨、特大鸭梨和油瓶 4 个地方品种独立出来为类群 4;当 K 为 5 时,魏县红梨表现出独特的遗传结构,被单独归为一类。当 K 为 6 时,笔者在 K 为 4 的基础上,进一步观察到 9 个河北其他地区的梨地方品种和魏县梨地方品种小白面被划分出了 3 个新的类群。通过交叉验证误差值(CV)分析,确定最佳 K 值为 3,因为此时的 CV 值最小,表明群体结构划分的最优解为 3 个群体。这一结果与主成分分析(PCA)的结果基本一致,进一步证实了其准确性。在 K 为 3 时,大部分魏县梨地方品种表现遗传背景单一,而魏县红梨、雪花和武安油秋的遗传背景相对

表3 测序数据及与参考基因组比对结果统计

Table 3 Statistics of sequencing and comparison of reference genome

样品 Sample	过滤后 reads 数 Clean reads	过滤后碱基数 Clean bases/bp	比对 reads 数 Mapped reads	比对碱基数 Mapped bases/bp	比对率 Mapping rate/%	平均测序深度 Average sequencing depth/X	覆盖率 Coverage/%
美香鸭梨 Meixiang Yali	426 267 86	639 401 790 0	419 137 06	616 926 371 4	98.33	10.66	95.85
白砣子 Baidunzi	446 748 46	670 122 690 0	438 003 21	642 708 323 1	98.04	10.40	95.42
大白面 Dabaimian	521 919 80	782 879 700 0	512 570 46	753 372 220 5	98.21	12.22	96.69
小白面 Xiaobaimian	557 495 08	836 242 620 0	548 790 10	808 077 732 0	98.44	12.78	96.33
鸭鸭面 Yayamian	516 377 42	774 566 130 0	506 599 13	744 431 500 2	98.11	11.85	96.15
小红面 Xiaohongmian	433 219 16	649 828 740 0	424 552 53	622 723 445 9	98.00	10.13	95.44
特大白面 Teda Baimian	529 357 70	794 036 550 0	519 729 87	763 823 167 4	98.18	12.38	96.21
油秋 Youqiu	508 666 76	763 000 140 0	498 698 46	731 837 229 7	98.04	11.56	95.72
武安油秋 Wu'an Youqiu	597 874 82	896 812 230 0	587 441 34	863 539 885 4	98.25	14.68	97.81
蒜白 Suanjiu	698 221 18	104 733 177 00	684 985 25	100 564 459 06	98.10	16.34	96.16
魏县红梨 Weixian Hongli	533 539 30	800 308 950 0	521 438 29	763 352 308 1	97.73	12.92	95.73
鸭梨 Yali	533 002 44	799 503 660 0	524 261 42	771 352 919 5	98.36	12.23	96.14
白雪花 Baixuehua	637 469 58	956 204 370 0	625 373 24	919 259 650 0	98.10	14.62	96.46
开心面 Kaixinmian	523 932 18	785 898 270 0	510 928 30	749 516 660 1	97.52	11.98	95.80
雪花 Xuehua	512 482 42	768 723 630 0	502 430 33	739 043 099 8	98.04	11.85	96.08
特大鸭梨 Teda Yali	430 623 28	645 934 920 0	423 470 49	623 089 322 2	98.34	10.19	95.78
金丰鸭梨 Jinfeng Yali	681 637 46	102 245 619 00	666 108 40	977 472 220 6	97.72	16.89	96.24
银白 Yinbai	413 562 10	620 343 150 0	406 144 10	597 010 486 8	98.21	10.22	95.74
白红梨 Baihongli	385 986 42	578 979 630 0	378 553 08	555 533 286 9	98.07	9.44	95.14
砣子梨 Dunzili	435 943 16	653 914 740 0	427 203 47	626 746 611 5	98.00	10.17	95.40
安梨 Anli	513 064 18	769 596 270 0	504 449 90	741 802 709 9	98.32	13.27	96.82
白花罐 Baihuaguan	539 581 20	809 371 800 0	529 465 54	777 669 102 0	98.13	12.31	94.78
白枝母秧 Baizhi Muyang	609 517 76	914 276 640 0	598 712 38	882 233 145 4	98.23	12.40	95.76
大紫酥 Dazisu	400 540 80	600 811 200 0	394 172 92	580 694 420 4	98.41	10.28	94.96
佛见喜 Fojianxi	764 130 40	114 619 560 00	750 367 79	110 413 758 31	98.20	19.45	96.08
红枝母秧 Hongzhi Muyang	489 861 76	734 792 640 0	481 957 30	709 650 374 7	98.39	10.97	95.65
鸡蛋罐 Jidanguan	448 763 24	673 144 860 0	441 334 03	649 651 702 1	98.34	10.30	95.00
金酥棠 Jinsutang	352 811 60	529 217 400 0	346 587 50	509 853 143 1	98.24	9.05	94.15
胎黄 Taihuang	577 439 56	866 159 340 0	567 790 04	836 230 617 2	98.33	14.04	95.63
油瓶 Youping	377 699 44	566 549 160 0	371 171 22	546 435 544 0	98.27	9.73	94.40

复杂(图3)。

对聚类分析、主成分分析(PCA)和 STRUCTURE 分析结果的综合比较,发现分析结果具有较高的相似性,互相印证。结果表明,魏县梨地方品种具有明显的聚类特征,群体遗传结构清晰,20个魏

县地方梨品种可划分为两大主要类群。值得注意的是,鸭梨、特大鸭梨和美香鸭梨3个地方品种在 PCA 和 STRUCTURE 分析中被归为亚群1,而在聚类分析中则被划分为亚群2,这一不同可能反映了不同分析方法对遗传结构解析的差异。

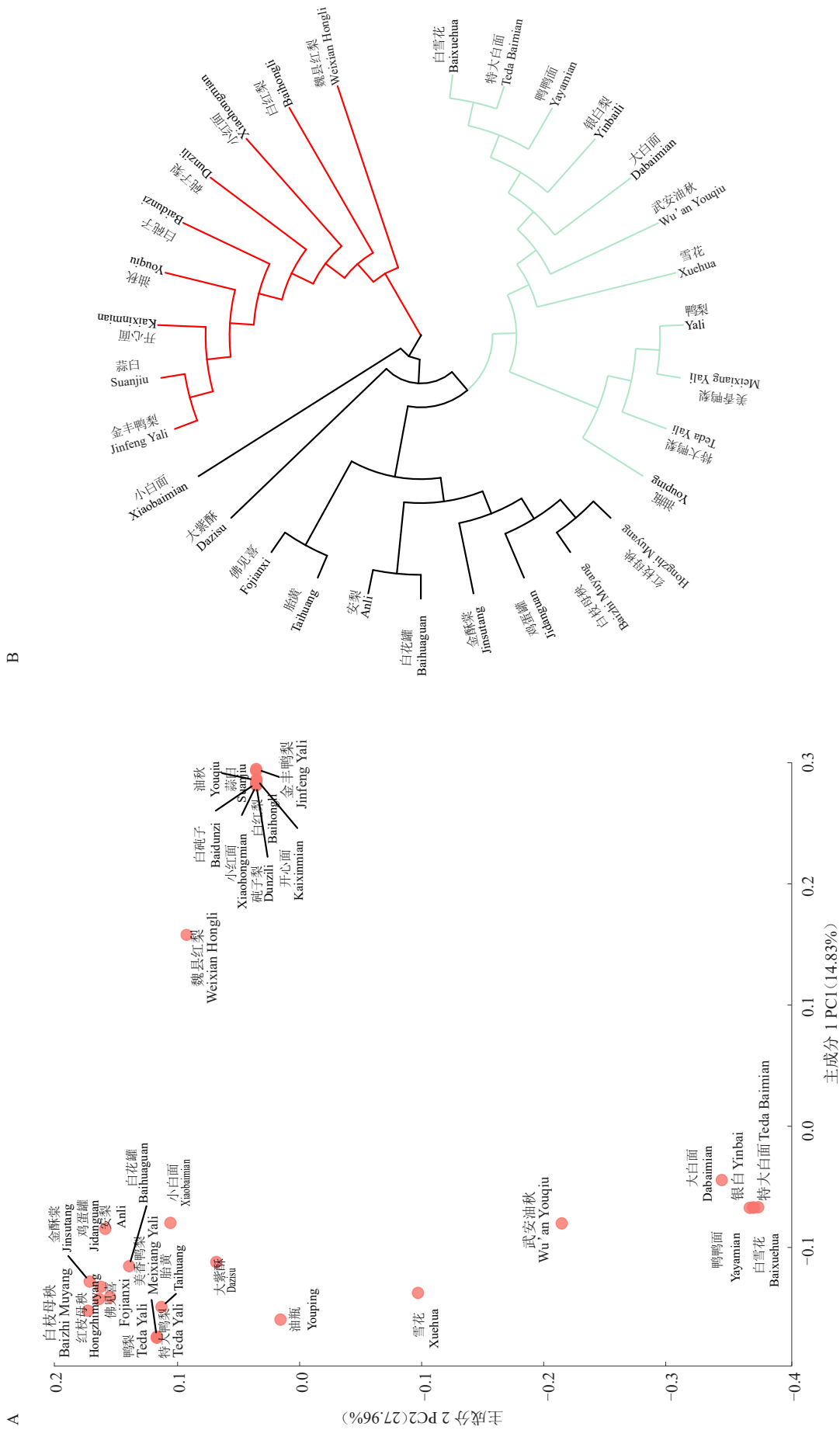
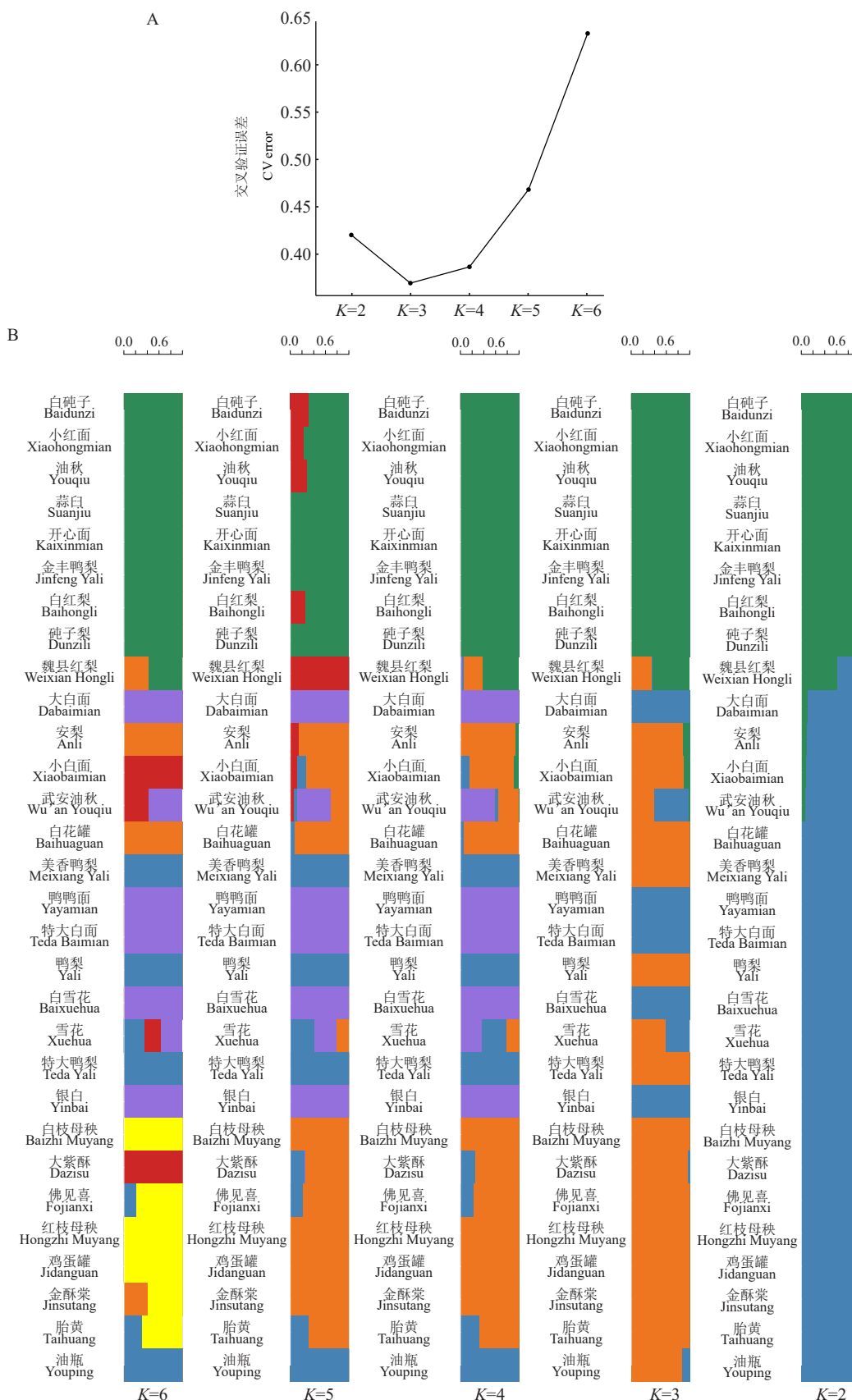


图 2 基于 SNPs 的主成分分析与系统发育树
 Fig. 2 Principal component analysis and phylogenetic tree based on SNPs



A. 基于 CV error 的结果确定最佳 K 值; B. K 值为 2~6 时的群体结构结果。

A. Determine the best K value based on the results of CV error; B. Population structure results when K is 2 to 6.

图3 基于SNP的群体结构分析

Fig. 3 Population structure analysis base on SNPs

3 讨 论

加强梨资源的保护和开发利用,对梨产业绿色发展具有重要意义^[30]。魏县梨栽培历史悠久,在长期的栽培过程中,魏县人民创造和保存了极具特色的梨地方品种。高通量测序的发展为梨种质资源的研究提供有力的技术支撑,Gao等^[31]采用基因分型测序(genotyping-by-sequencing, GBS)技术对安徽梨地方品种进行了亲缘关系分析和群体遗传结构解析,在81个梨种质资源,其中安徽梨地方品种48个,共鉴定出242447全基因组范围的SNPs位点。本研究利用重测序技术在30个梨种质资源中鉴定出高质量的SNPs 300 418 77个,标记密度和覆盖度得到显著提升。魏县地方梨品种可以划为两大类群,类群1包含魏县红梨、油秋等9个地方品种,类群2包含11个地方品种,进一步划分为2个亚群,亚群1包括小白面这1个地方品种,亚群2由雪花、武安油秋等7个地方品种组成;此外,类群2还包括10个起源于河北其他地区的秋子梨品种和白梨品种,其中安梨和白花罐为典型的北方秋子梨品种,其他地方品种的原产地为河北北部的青龙、兴隆等地区。由此可见,类群2的魏县梨地方品种与河北其他地区梨品种间具有更广泛的基因交流,鉴于魏县位于河北最南端,推测该类群的魏县梨地方品种与北方梨品种群的亲缘关系近。类群1的9个魏县梨地方品种,与北方梨品种群的亲缘关系较远,其中的魏县红梨属于砂梨品种,果皮为红褐色,叶绿体单倍型与其他品种的单倍型不同,推测该类群与南方品种群的亲缘关系更近,魏县梨地方品种同南方梨品种群也存在基因交流。

芽变育种是果树新品种选育的重要途径,对于果树育种来说,芽变选种主要是对重要品质性状的优中选优^[32],同时芽变品种可作为相关果实品质基因鉴定的重要材料,为果实品质相关基因提供材料基础^[33]。利用表型性状可对果树芽变品种进行初步的鉴定,前人记载美香鸭梨、金丰鸭梨和特大鸭梨为鸭梨的芽变品种^[2]。美香鸭梨果面光洁果点少,金丰鸭梨具有自花结实率高的特性,特大鸭梨是鸭梨的大果型变异。SSR标记作为梨品种鉴定的常用方法,因其开发的位点数量有限,对梨芽变品种的鉴定较为困难,曹玉芬等^[34]的研究发现12对SSR引物对鸭梨及其芽变品种晋县大鸭梨和垂枝鸭梨扩增等位基因相同。笔者在本研究中的聚类分析显示,鸭梨、

美香鸭梨和特大鸭梨归属于同一分支,且叶绿体单倍型相同,在分子水平上证实美香鸭梨和特大鸭梨是鸭梨的芽变,与表型性状的鉴定结果一致^[2];金丰鸭梨的聚类结果和单倍型均与上述3个鸭梨品种存在差异,金丰鸭梨与蒜白梨的亲缘关系更近,且两者叶绿体单倍型相同,对比美香鸭梨和特大鸭梨的聚类结果和叶绿体单倍型信息,推断金丰鸭梨并非鸭梨的芽变品种,或为鸭梨的实生选育品种。砣子梨和白砣子的遗传背景相似,亲缘关系近,证实白砣子梨是砣子梨的一个芽变品种。

白雪花和雪花在同一类群,有别于雪花,白雪花的果肉稍细,两者的叶绿体单倍型结果不一致,可以判断白雪花不是雪花的芽变,聚类分析等结果显示白雪花与特大白面的亲缘关系更近,遗传背景相似。油秋与武安油秋在聚类分析和PCA分析中被划分到不同类群,STRUCTURE分析结果也显示,两者的遗传背景存在显著差异,油秋更接近北方梨品种群,武安油秋更接近南方梨品种群,因此这些品种尽管品种名称相似,但亲缘关系较远。白红梨被认为是银白和魏县红梨的杂交实生类型或魏县红梨的芽变,笔者在本研究中发现白红梨与魏县红梨的叶绿体单倍型不一致,但与其亲缘关系近,推测魏县红梨是其父本的可能性较大。开心面与小红面具有相同叶绿体单倍型,且与小红面、砣子梨在同一类群,可能为二者杂交选育获得。

4 结 论

基于全基因组重测序揭示的单核苷酸多态性(SNPs)及叶绿体DNA变异信息,明确了魏县梨地方品种的亲缘关系和群体遗传结构,研究结果为魏县梨品种的遗传改良提供新的见解和信息,同时美香鸭梨、特大鸭梨、白砣子等芽变材料将为果实品质相关基因的鉴定提供重要的材料基础。

参考文献 References:

- [1] 李振江,李云峰,贾艳利,李小虎,申晓峰,王永博. 晚熟梨新品种‘美香’[J]. 园艺学报, 2024, 51(增刊 1): 23-24.
LI Zhenjiang, LI Yunfeng, JIA Yanli, LI Xiaohu, SHEN Xiaofeng, WANG Yongbo. A new late-maturing pear cultivar ‘Meixiang’ [J]. Acta Horticulturae Sinica, 2024, 51(Suppl. 1): 23-24.
- [2] 刘振廷,冯立学. 中国魏县梨品种集[M]. 北京: 中国农业科学技术出版社, 2018.
LIU Zhenjing, FENG Lixue. Collection of pear varieties from

- Weixian[M]. China Agricultural Science and Technology Press, 2018.
- [3] XUE L, LIU Q W, HU H J, SONG Y, FAN J, BAI B, ZHANG M Y, WANG R Z, QIN M F, LI X L, WU J. The southwestern origin and eastward dispersal of pear (*Pyrus pyrifolia*) in East Asia revealed by comprehensive genetic structure analysis with SSR markers[J]. *Tree Genetics & Genomes*, 2018, 14(4):48.
- [4] OUNI R, ZBOROWSKA A, SEHIC J, CHOULAK S, HORMAZA J I, GARKAVA-GUSTAVSSON L, MARS M. Genetic diversity and structure of Tunisian local pear germplasm as revealed by SSR markers[J]. *Horticultural Plant Journal*, 2020, 6(2): 61-70.
- [5] ZURN J D, NYBERG A, MONTANARI S, POSTMAN J, NEALE D, BASSIL N. A new SSR fingerprinting set and its comparison to existing SSR- and SNP- based genotyping platforms to manage *Pyrus* germplasm resources[J]. *Tree Genetics & Genomes*, 2020, 16(5): 72.
- [6] 刘珊廷, 易显荣, 周民武, 吴潇, 齐开杰, 徐志美, 赵碧英. 广西梨种质资源遗传多样性和群体结构分析[J]. *果树学报*, 2024, 41(3):379-391.
LIU Shanting, YI Xianrong, ZHOU Minwu, WU Xiao, QI Kaijie, XU Zhimei, ZHAO Biying. Analysis of genetic diversity and population structure of pear germplasm resources in Guangxi[J]. *Journal of Fruit Science*, 2024, 41(3):379-391.
- [7] XUE H B, ZHANG P J, SHI T, YANG J, WANG L, WANG S K, SU Y L, ZHANG H R, QIAO Y S, LI X G. Genome-wide characterization of simple sequence repeats in *Pyrus bretschneideri* and their application in an analysis of genetic diversity in pear[J]. *BMC Genomics*, 2018, 19(1):473.
- [8] 蒋爽, 骆军, 王晓庆, 施春晖. 基于基因组重测序数据高效筛选梨 SSR 标记多态性引物[J]. *果树学报*, 2019, 36(2):129-136.
JIANG Shuang, LUO Jun, WANG Xiaoqing, SHI Chunhui. A study on efficient screening of the primers for selecting polymorphic SSR markers based on the re-sequencing data in *Pyrus*[J]. *Journal of Fruit Science*, 2019, 36(2):129-136.
- [9] 王富强, 樊秀彩, 张颖, 刘崇怀, 姜建福. SNP 分子标记在作物品种鉴定中的应用和展望[J]. *植物遗传资源学报*, 2020, 21(5):1308-1320.
WANG Fuqiang, FAN Xiucui, ZHANG Ying, LIU Chonghuai, JIANG Jianfu. Application and prospect of SNP molecular markers in crop variety identification[J]. *Journal of Plant Genetic Resources*, 2020, 21(5): 1308-1320.
- [10] WU J, WANG Z W, SHI Z B, ZHANG S, MING R, ZHU S L, AWAIS KHAN M, TAO S T, KORBAN S S, WANG H, CHEN N J, NISHIO T, XU X, CONG L, QI K J, HUANG X S, WANG Y T, ZHAO X, WU J Y, DENG C, GOU C Y, ZHOU W L, YIN H, QIN G H, SHA Y H, TAO Y, CHEN H, YANG Y N, SONG Y, ZHAN D L, WANG J, LI L T, DAI M S, GU C, WANG Y Z, SHI D H, WANG X W, ZHANG H P, ZENG L, ZHENG D M, WANG C L, CHEN M S, WANG G B, XIE L, SOVERO V, SHA S F, HUANG W J, ZHANG S J, ZHANG M Y, SUN J M, XU L L, LI Y, LIU X, LI Q S, SHEN J H, WANG J Y, PAULL R E, BENNETZEN J L, WANG J, ZHANG S L. The genome of the pear (*Pyrus bretschneideri* Rehd.) [J]. *Genome Research*, 2013, 23(2):396-408.
- [11] DONG X G, WANG Z, TIAN L M, ZHANG Y, QI D, HUO H L, XU J Y, LI Z, LIAO R, SHI M, ALI WAHOCHO S, LIU C, ZHANG S M, TIAN Z X, CAO Y F. *De novo* assembly of a wild pear (*Pyrus betuleafolia*) genome[J]. *Plant Biotechnology Journal*, 2020, 18(2):581-595.
- [12] LINSMITH G, ROMBAUTS S, MONTANARI S, DENG C H, CELTON J M, GUÉRIF P, LIU C, LOHAUS R, ZURN J D, CESTARO A, BASSIL N V, BAKKER L V, SCHIJLEN E, GARDINER S E, LESPINASSE Y, DUREL C E, VELASCO R, NEALE D B, CHAGNÉ D, VAN DE PEER Y, TROGGIO M, BIANCO L. Pseudo-chromosome-length genome assembly of a double haploid “Bartlett” pear (*Pyrus communis* L.) [J]. *GigaScience*, 2019, 8(12):giz138.
- [13] OU C Q, WANG F, WANG J H, LI S, ZHANG Y J, FANG M, MA L, ZHAO Y N, JIANG S L. A *de novo* genome assembly of the dwarfing pear rootstock Zhongai 1[J]. *Scientific Data*, 2019, 6(1):281.
- [14] SUN M Y, YAO C J, SHU Q, HE Y Y, CHEN G S, YANG G Y, XU S Z, LIU Y Y, XUE Z L, WU J. Telomere-to-telomere pear (*Pyrus pyrifolia*) reference genome reveals segmental and whole genome duplication driving genome evolution[J]. *Horticulture Research*, 2023, 10(11):uhad201.
- [15] 张明月. 基于基因组学的梨群体遗传变异研究[D]. 南京:南京农业大学, 2017.
ZHANG Mingyue. Study of genetic variation by genomics in pear population[D]. Nanjing: Nanjing Agricultural University, 2017.
- [16] WU J, WANG Y T, XU J B, KORBAN S S, FEI Z J, TAO S T, MING R, TAI S S, KHAN A M, POSTMAN J D, GU C, YIN H, ZHENG D M, QI K J, LI Y, WANG R Z, DENG C H, KUMAR S, CHAGNÉ D, LI X L, WU J Y, HUANG X S, ZHANG H P, XIE Z H, LI X, ZHANG M Y, LI Y H, YUE Z, FANG X D, LI J M, LI L T, JIN C, QIN M F, ZHANG J Y, WU X, KE Y Q, WANG J, YANG H, ZHANG S L. Diversification and independent domestication of Asian and European pears[J]. *Genome Biology*, 2018, 19(1):77.
- [17] 高雨豪. 梨属植物基因组的进化与遗传变异分析[D]. 杭州:浙江大学, 2023.
GAO Yuhao. Analysing evolution and genetic variations of *Pyrus* genomes[D]. Hangzhou: Zhejiang University, 2023.
- [18] 郑小艳, 滕元文. 基于多种 DNA 序列的霉梨起源初探[J]. *园艺学报*, 2014, 41(10):2107-2114.
ZHENG Xiaoyan, TENG Yuanwen. Origin of meili (*Pyrus* L.) based on data from multiple DNA sequences[J]. *Acta Horticulturae Sinica*, 2014, 41(10):2107-2114.

- [19] CHANG Y J, CAO Y F, ZHANG J M, TIAN L M, DONG X G, ZHANG Y, QI D, ZHANG X S. Study on chloroplast DNA diversity of cultivated and wild pears (*Pyrus L.*) in Northern China[J]. *Tree Genetics & Genomes*, 2017, 13(2):44.
- [20] 齐丹,常耀军,曹玉芬,胡红菊,田路明,董星光,张莹,霍宏亮,徐家玉,张小双,刘超. 基于叶绿体 DNA 信息的南方梨属种质的遗传多样性和演化分析[J]. *园艺学报*, 2018, 45(12):2308-2320.
- QI Dan, CHANG Yaojun, CAO Yufen, HU Hongju, TIAN Luming, DONG Xingguang, ZHANG Ying, HUO Hongliang, XU Jiayu, ZHANG Xiaoshuang, LIU Chao. Genetic diversity and phylogenetics of pear (*Pyrus L.*) germplasm resources from South China revealed by chloroplast DNA[J]. *Acta Horticulturae Sinica*, 2018, 45(12):2308-2320.
- [21] YUE X Y, ZHENG X Y, ZONG Y, JIANG S, HU C Y, YU P Y, LIU G Q, CAO Y F, HU H J, TENG Y W. Combined analyses of chloroplast DNA haplotypes and microsatellite markers reveal new insights into the origin and dissemination route of cultivated pears native to east Asia[J]. *Frontiers in Plant Science*, 2018, 9:591.
- [22] KUMAR S, STECHER G, LI M, KNYAZ C, TAMURA K. MEGA X: Molecular evolutionary genetics analysis across computing platforms[J]. *Molecular Biology and Evolution*, 2018, 35(6):1547-1549.
- [23] LIBRADO P, ROZAS J. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data[J]. *Bioinformatics*, 2009, 25(11):1451-1452.
- [24] BANDEL T H J, FORSTER P, RÖHL A. Median-joining networks for inferring intraspecific phylogenies[J]. *Molecular Biology and Evolution*, 1999, 16(1):37-48.
- [25] LI H, DURBIN R. Fast and accurate short read alignment with Burrows-Wheeler transform[J]. *Bioinformatics*, 2009, 25(14):1754-1760.
- [26] LI H, HANDSAKER B, WYSOKER A, FENNEL T, RUAN J, HOMER N, MARTH G, ABECASIS G, DURBIN R, 1000 Genome Project Data Processing Subgroup. The sequence alignment/map format and SAMtools[J]. *Bioinformatics*, 2009, 25(16):2078-2079.
- [27] MCKENNA A, HANNA M, BANKS E, SIVACHENKO A, CIBULSKIS K, KERNYTSKY A, GARIMELLA K, ALT-SHULER 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.
- [28] ALEXANDER D H, NOVEMBRE J, LANGE K. Fast model-based estimation of ancestry in unrelated individuals[J]. *Genome Research*, 2009, 19(9):1655-1664.
- [29] YANG J, LEE S H, GODDARD M E, VISSCHER P M. GCTA: A tool for genome-wide complex trait analysis[J]. *The American Journal of Human Genetics*, 2011, 88(1):76-82.
- [30] 张绍铃,谢智华. 我国梨产业发展现状、趋势、存在问题与对策建议[J]. *果树学报*, 2019, 36(8):1067-1072.
- ZHANG Shaoling, XIE Zhihua. Current status, trends, main problems and the suggestions on development of pear industry in China[J]. *Journal of Fruit Science*, 2019, 36(8):1067-1072.
- [31] GAO Z H, MA N, QI Y J, KAN L P, XU Y L. Genetic relationships and population structure of pear accessions from Anhui, China, based on genotyping-by-sequencing[J]. *Plant Molecular Biology Reporter*, 2025, 43(1):216-226.
- [32] 黄奕琦,朱玉琴,杜肇轩,徐丰,陈小怡,杨国顺,许延帅. 果树芽变机制研究进展[J]. *园艺学报*, 2024, 51(7):1547-1564.
- HUANG Yiqi, ZHU Yuqin, DU Zhaoxuan, XU Feng, CHEN Xiaoyi, YANG Guoshun, XU Yanshuai. Progress on bud sport of tree fruit[J]. *Acta Horticulturae Sinica*, 2024, 51(7):1547-1564.
- [33] 沈辉,殷亚蕊,武军凯,王海静,黄佳,左波,张立彬,于凤鸣. ‘鸭梨’及其自交亲和性突变体‘金坠梨’花粉蛋白质组比较分析[J]. *果树学报*, 2019, 36(9):1101-1111.
- SHEN Hui, YIN Yarui, WU Junkai, WANG Haijing, HUANG Jia, ZUO Bo, ZHANG Libin, YU Fengming. Comparative proteome analysis of pollens from ‘Yali’ pear and its self-compatible mutant, ‘Jinzhu’ pear[J]. *Journal of Fruit Science*, 2019, 36(9):1101-1111.
- [34] 曹玉芬,刘凤之,高源,姜立杰,王昆,马智勇,张开春. 梨栽培品种 SSR 鉴定及遗传多样性[J]. *园艺学报*, 2007, 34(2):305-310.
- CAO Yufen, LIU Fengzhi, GAO Yuan, JIANG Lijie, WANG Kun, MA Zhiyong, ZHANG Kaichun. SSR analysis of genetic diversity of pear cultivars[J]. *Acta Horticulturae Sinica*, 2007, 34(2):305-310.