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基于SSR标记技术的南丰柑橘种质资源亲缘关系研究

辜青青[1a],曾 涛[2a],魏清江[1],邹菊花[1],陈金印[1*]

([1]江西农业大学农学院,南昌 330045;[2]新余市果业局,江西新余 338000)

摘 要:【目的】从分子水平探究南丰柑橘种质间的亲缘关系。【方法】应用SSR技术对28份南丰柑橘种质进行基因组的多态性分析。【结果】从91对引物中筛选得到13对SSR引物,可区分全部供试材料。共扩增得到64个等位基因,平均每个位点4.92个等位基因。以遗传相似系数0.18为界,金柑属金柑单独聚为1类,柑橘属27份供试样品聚为1类。以相似系数0.59为阈值,27份柑橘属供试种质分为5个类群,其中南丰蜜橘品种群(包含小果系、大果系、桂花蒂系、早熟系)所有20份供试种质与'蜜广'聚为一类,'小叶广''火广''火橘'聚为一类,'红广''红橘''本地早橘'各为一类。【结论】南丰广橘品种群中'蜜广'与南丰蜜橘品种群有较近的亲缘关系,而'红广'与亲本南丰蜜橘和'红橘'的亲缘关系均较远。'小叶广''火广'和'火橘'亲缘关系较近。

关键词:南丰蜜橘品种群;南丰广橘品种群;微卫星标记;亲缘关系

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Genetic relationship analysis among Nanfeng citrus using SSR markers

GU Qingqing[1a], ZENG Tao[2a], WEI Qingjiang[1], ZOU Juhua[1], CHEN Jinyin[1*]

(*[1]College of Agronomy, Jiangxi Agricultural University, Nanchang 330045, Jiangxi, China; [2]Bureau of Xinyu Fruit Industry, Xinyu 338000, Jiangxi, China*)

Abstract:【Objective】Nanfeng tangerines (*Citrus reticulate* Blanco 'Kinokuni') are one of the most famous local cultivars of China. It has more than 1 300 years of cultivation history. Due to the high frequency of natural variation and natural hybridization, the Nanfeng tangerine gradually formed the Nanfeng tangerine group (such as small-fruit Nanfeng tangerines, large-fruit Nanfeng tangerines, early-ripe Nanfeng tangerines, 'Guihuadi' Nanfeng tangerines, etc.) and the Nanfeng guangju group (such as 'Hongguang' 'Miguang' etc.). Based on the comprehensive collection of Nanfeng citrus germplasms, this study utilized SSR markers to explore their genetic relationships from the molecular level, in order to provide a theoretical basis for the Nanfeng citrus germplasm resources preservation and utilization.【Methods】Twenty-eight germplasm accessions of Nanfeng citrus were used as materials for analyzing their genome polymorphism, including 15 small-fruit Nanfeng tangerine cultivars, two large-fruit Nanfeng tangerine cultivars, two early-ripe Nanfeng tangerine cultivars, 'Guihuadi' Nanfeng tangerine, 'Hongguang' 'Miguang' 'Xiaoyeguang' 'Huoguang' 'Red tangerine' 'Huoju' 'Bendizaoju' and kumquat all collected from the Nanfeng Tangerine Germplasm Repository in Nanfeng county, Shuinan and Dabao village in Qincheng town, Nanfeng county, and the Nanfeng Tangerine Germplasm Repository in Horticultural Sciences Institute of Jiangxi Agricultural Sciences Academy. The collected fresh leaves were brought back to the laboratory in

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作者简介:辜青青,女,副教授,硕士,研究方向为果树种质资源与品质调控。Tel:13970947076,E-mail: qingqinggu2006@126.com。a为共同第一作者,曾涛,男,助理研究员,硕士。Tel:15907904370,E-mail: zt191919@163.com

*通信作者 Author for correspondence. E-mail: jinyinchen@126.com

iced cassettes. After their surfaces were cleaned by clear water, they were rapidly frozen in liquid nitrogen and stored at −80 ℃ until required for testing. The improved CTAB method was used to extract the total DNA genome, 91 pairs of SSR primers were used from previous reports. The total PCR reaction volume was 25 μL: 2×*Taq* PCR MasterMix 12.5 μL, 10 μmol·L^{-1} forward and reverse primers each 0.5 μL, 50 mg·L^{-1} template DNA 0.5 μL, ddH_2O 11 μL. The cycles were programmed as follows: one initial denaturing cycle at 94 ℃ for 4 min, 32 cycles of 30 s denaturing at 94 ℃, 45 s annealing at 45−60 ℃, 45 s elongation at 72 ℃ and one final cycle of 5 min at 72 ℃, stored at 4 ℃. The products were electrophoresised on 6% (ρ) denaturing polyacrylamide gel and visualized using silver staining. The polymorphism was determined according to the presence (scored as "1") or absence (scored as "0") of the SSR band. The similarity coefficient was calculated using NTSYS−pc2.1 software. UPGMA (unweighted pair group method arithmetic averages) were determined by using clustering analysis. The polymorphic information content (PIC) values for each marker were calculated according to the following formula: PIC = 1−ΣP_{ij}^2, where P_{ij} is the frequency of the jth pattern for SSR marker i. 【Results】Thirteen out of the 91 SSR loci were used for genotpying and they were able to distinguish all accessions. Sixty−four alleles were identified across all loci, with an average of 4.92 alleles per locus. UPGMA analysis showed that the kumquat (*Fortunella crassifolia* Swing.) was separated from the other 27 accessions of *Citrus* at the similarity coefficient of 0.18. At the coefficient of 0.59, the remaining 27 accessions of *Citrus* were divided into five major groups. All the 20 Nanfeng tangerine cultivars and Miguang could be clustered into one group (Ⅰ), and 'Xiaoyeguang' 'Huoguang' and 'Huoju' were clustered into another group (Ⅱ). 'Hongguang' 'Red tangerine' and 'Bendizao tangerine' were distributed in group Ⅲ, Ⅳ and Ⅴ, respectively. When the similarity coefficient was 0.70, group Ⅰ was divided into 2 subgroups. 'Guihuadi' Nanfeng tangerine, early−ripe Nanfeng tangerine 'LS−1' and 14 small−fruit Nanfeng tangerine cultivars were distributed in the Ⅰ−1 subgroup. The biological characteristics and economic traits of 'Guihuadi' Nanfeng tangerine and early−ripe Nanfeng tangerine 'LS−1' were fundamentally the same as the small−fruit Nanfeng tangerine except for sepals like osmanthus flower shape and an early mature period of 7−10 d, respectively. Small−fruit Nanfeng tangerine '97−2', early−ripe Nanfeng tangerine '97−1', large−fruit Nanfeng tangerine 'LS−1', large−fruit Nanfeng tangerine '97−1' and 'Miguang' were distributed in subgroup Ⅰ−2. Each of the Nanfeng tangerine strains did not separate during clustering. However, the small−fruit Nanfeng tangerine was primarily concentrated in subgroup Ⅰ−1.【Conclusion】The genetic relationships between the 'Miguang' and Nanfeng tangerine were closer than that between the 'Hongguang' and Nanfeng tangerine. In addition, 'Xiaoyeguang' 'Huoguang' and 'Huoju' were clustered together, indicating they have close genetic relationships.

Key words: Nanfeng tangerine; Nanfeng guangju; SSR marker; Genetic relationship

南丰蜜橘源出乳橘,人们以其味甜如蜜便称之为蜜橘,继而又冠以地名传称南丰蜜橘,是我国著名的柑橘地方良种之一,栽培史可追溯到唐代开元之前,至少有1 300年以上[1]。明代以后,南丰蜜橘生产渐次兴盛,由于高频率的自然变异及自然杂交等原因,逐渐形成由新品系(如小果系、大果系、桂花蒂系、早熟系等)构成的南丰蜜橘品种群及南丰广橘品种群。南丰广橘,群众称假蜜橘,又称鸳鸯蜜橘,分'蜜广'和'红广'2大类群,是南丰蜜橘主产区的另一品种群。'蜜广',系以南丰蜜橘为母本,以'火橘'(又称'黄皮柑')为父本的花粉蒙导的杂交种;'红广',系以南丰蜜橘为母本,以当地'红橘'(又称'小叶红橘')为父本的自然杂交种[2]。'火橘'为南丰主产,戴麟1935年在南丰进行实地调查后认为橘农以其采收前味辣如火酒,故名'火橘'[1]。

SSR(simple sequence repeats)为共显性标记,所需样品DNA量少,多态性高,操作相对简单,PCR产物稳定性和重复性好,已被广泛用于苹果[3]、葡萄[4]、