ACTA AGRICULTURAE BOREALI-SINICA 华北农学报• 2008, 23(增刊): 129-132

分子标记技术在咖啡育种上的应用

李国鹏, 何红艳, 黄家雄, 罗心平, 李贵平, 杨仕贵, 李树维, 黄 健, 郭容琦

(云南省农科院 热带亚热带经济作物研究所, 云南 保山 678025)

摘要: 主要介绍了AFLP和RAPD 2种分子标记技术以及2种分子标记的优缺点。同时, 着重介绍了2种分子标记在咖啡育种上的应用成果。在最后还列举了2个其他的分子标记在咖啡育种上的应用。

关键词: 咖啡; 分子标记技术; AFLP; RAPD; 应用

中图分类号: Q75 文献标识码: A 文章编号: 1000-7091(2008)增刊-0129-04

Molecule Marker Technology in Coffee Breeding Application

LI Guo-peng, HE Hong-yan, HUANG Jia-xiong, LUO Xin-ping, LI Gui-ping,
YANG Shi-gui, LI Shu-wei, HUANG Jian, GUO Rong-qi
(Tropical and Subtropical Cash Crops Institute of Yunnan Agriculture
Science Research Institute, Baoshan 678025, China)

Abstract: This article introduced AFLP and RAPD technologies, and its application in coffee breeding program. This article introduced the merit and disadvantages of the AFLP and RAPD. At the end, gave 2 samples of other molecule marker technology in coffee breeding.

Key words: Coffee; Molecule marker technology; AFLP; RAPD; Application

Coffee and tea are the two most important beverages of the world. Although the genus Coffee contains about 80 taxa, commercial coffee production is based on two species i.e. *C. arabica* (2n= 4x= 44) and *C. canephora* (2n= 2x= 22). They accounting respectively for about 70% and 30% of the world production. The Arabica coffee cultivation started in Arabia, specifically in Yemen, five centuries ago. In the early 18th century, progenies from a single Indonesia plant cultivated in Europe were spread out to South America and turned out to be the genetic basis of main cultivars of Brazil and other countries. Several centuries ago, the aim of crop breeding just emphasis on high yield. Nowadays, the breeding program are centering on high quality and selecting new cultivars that resistant to coffee leaf rust. The traditional breeding method is just hand worked crossing. It has many defects, such as need long time to observe the offspring; sensitive to environmental factors.

In recent years, DNA molecular markers such as AFLP, RAPD and ISSR, which are independent of environmental influences, have been used in the coffee breeding research. In this article, we just introduced the principles of some modern biotechnologies and its application on the coffee breeding research.

1 The brief introduction and utilization of AFLP in coffee breeding

Amplified Fragment Length Polymorphisms (AFLP) is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA. And AFLP is a DNA fingerprint analysis method with high discriminatory capacity. Compared to other marker techniques, AFLP is more efficient, fast, reliable and highly polymorphic. The power of AFLP is based upon the molecular genetic variations that exist between closely related species, varieties or cultivars. These variations in DNA sequence

收稿日期: 2008-08-02
基金项目: 农业部农业公益性行业科研专项(200803045); 农业厅创新办"小粒咖啡GAP栽培技术集成与示范"项目
作者简介: 李国鹏(1982-), 男, 河北井陉人, 硕士, 研究实习员, 主要从事热带作物研究。
通讯作者: 何红艳(1983-), 女, 云南禄丰人, 学士, 主要从事热带作物和咖啡研究。

are exploited by the AFLP technology such that "fingerprints" of particular genotypes can be routinely generated. These "fingerprints" are simply RFLPs visualized by selective PCR amplification of DNA restriction fragments. There are several weaknesses of AFLP: ①Proprietary technology is needed to score heterozygote. Otherwise, AFLP must be dominantly scored. ②Developing locus-specific markers from individual fragments can be difficult. ③Need to use different kits adapted to the size of the genome being analyzed.

Lashermes P. etc[1] evaluated the Timor Hybrid-derived genotypes by AFLP, using 42 different primer combinations, and compared to 23 accessions of *C. arabica* and 8 accessions of *C. canephora*. A total of 1 062 polymorphic fragments were scored among the 52 accessions analysed. One hundred and seventy-eight markers consisting of 109 additional bands (i. e. introgressed markers) and 69 missing bands distinguished the group composed of the Timor Hybrid-derived genotypes from the accessions of *C. arabica*. The genetic diversity observed in the Timor Hybrid-derived genotypes appeared to be approximately double that in *C. arabica*. Although representing only a small proportion of the genetic diversity available in *C. canephora*, the Timor Hybrid obviously constitutes a considerable source of genetic diversity for Arabica breeding. Analysis of genetic relationships among the Timor Hybrid-derived genotypes suggested that introgression was not restricted to chromosome substitution but also involved chromosome recombinations. Furthermore, the Timor Hybrid-derived genotypes varied considerably in the number of AFLP markers attributable to introgression. In this way, the introgressed markers identified in the analysed arabica coffee introgressed genotypes were estimated to represent from 9% to 29% of the *C. canephora* genome. Nevertheless, the amount of alien genetic material in the introgression arabica lines remains substantial and should justify the development of adapted breeding strategies.

Mahe L. etc[2] used twelve AFLP primer combinations in their study. A total of 250 AFLP fragments were scored in the group of HNC plants. All the AFLP fragments observed in C. *arabica* accessions were identified within the group of HNC plants. On the other hand, 129 AFLP fragments not detected in any of the accessions of C. *arabica* were observed. Those markers were therefore considered further as *C. canephora* markers. Although the number of C. *canephora* clones included in this study was limited, 115 of these 129 marker bands were also observed in at least one of the Canephora samples analyzed, confirming their Canephora origin. Furthermore, they found that the total number of Canephora markers varied from 2 to 60 among the HNC plants. Thus, the introgressed fraction of C. *canephora* genome as estimated through the AFLP introgression index appeared highly variable. While six plants are likely to be F1 hybrids (i. e. introgression index of 1), the plant L8 displayed a low introgression rate (i. e. 4%).

Anthony F. etc[3,4] used molecular markers to assess polymorphism between and within the genetic bases of coffee (i. e. Typica and Bourbon) spread from Yemen since the early 18th century. Eleven *Coffea arabica* accessions derived from the disseminated bases were evaluated by AFLP using 37 primer combinations. One hundred and seven AFLP markers were used to calculate genetic distances and construct a dendrogram. The accessions derived from the disseminated bases were grouped separately, according to their genetic origin, and were distinguished from the subspontaneous accessions. The Yemen cultivars were classified with the Typica-derived accessions. Except for one AFLP marker, all AFLP markers present in the cultivated accessions were also detected in the subspontaneous accessions. Polymorphism among the subspontaneous accessions was much higher than among the cultivated accessions. It was very low within the genetic bases, confirming the historical documentation on their dissemination. The results enabled a discussion of the genetic diversity reductions that successively occurred during the dissemination of *C. arabica* from its primary centre of diversity.

2 The brief introduction to and utilization of RAPD in coffee breeding

Compared with various molecular maker techniques, RAPD (Random Amplified Polymorphic DNA) has a lot of advantages such as abundant polymorphism, high sensitivity, and economical, rapid and convenient operation requiring only a small quantity of DNA and no special probe which is extensively used in genetic mapping and gene localization. RAPD markers are DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence. Unlike traditional PCR analysis, RAPD does not require any specific knowledge of the DNA sequence of the target organism:

the identical 10-mer primers will or will not amplify a segment of DNA, depending on positions that are complementary to the primers' sequence. For example, no fragment is produced if primers annealed too far apart or 3′ ends of the primers are not facing each other. Therefore, if a mutation has occurred in the template DNA at the site that was previously complementary to the primer, a PCR product will not be produced, resulting in a different pattern of amplified DNA segments on the gel. Limitations of RAPD: ①Nearly all RAPD markers are dominant, i. e. It is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous (1 copy) or homozygous (2 copies). Co-dominant RAPD markers, observed as different-sized DNA segments amplified from the same locus, are detected only rarely. ②PCR is an enzymatic reaction, therefore the quality and concentration of template DNA, concentrations of PCR components, and the PCR cycling conditions may greatly influence the outcome. Thus, the RAPD technique is notoriously laboratory dependent and needs carefully developed laboratory protocols to be reproducible. ③Mismatches between the primer and the template may result in the total absence of PCR product as well as in a merely decreased amount of the product. Thus, the RAPD results can be difficult to interpret.

Anthony F. etc[5] studied genetic diversity using RAPD markers among 119 coffee (*Coffea arabica* L.) individuals representing 88 accessions derived from spontaneous and subspontaneous trees in Ethiopia, the primary centre of species diversity, six cultivars grown locally in Ethiopia, and two accessions derived from the genetic populations Typica and Bourbon, spread in the 18th century, which gave rise to the most currently grown cultivars. Twenty-nine polymorphic fragments were used to calculate a similarity index and construct dendrograms. The Ethiopian material was separated from the Typica- and Bourbon-derived accessions and classified in four groups: one with most of the collected material from southwestern Ethiopia and three from southern and southeastern Ethiopia. Almost all detected diversity was found in the southwestern group while the southern and southeastern groups presented only 59% of identified markers. The genetic distances were low between the southwestern group and the southern and southeastern groups, and between the southwestern group and the Typica- and Bourbon-derived accessions. The cultivated coffee derived from the genetic populations Typica and Bourbon appeared little differentiated from wild coffee growing in the southwest. The results supported the hypothesis that southwestern Ethiopian coffee trees could have been introduced recently in the south and southeast. A separate analysis of the 80 accessions classified in the southwestern group allowed identifying particular spontaneous and subspontaneous-derived accessions and redundancies in the collected material from southwestern Ethiopia. RAPD markers did not detect any within-collection polymorphismexcept for two trees that were identified as off-types in the CATIE field genebank.

RAPD analysis was performed to estimate the level of genetic diversity within the germplasm collection and the relatedness between cultivated and subspontaneous accessions of C. *arabica*. Six varieties representing the two distinct cultivated coffee types (typica and bourbon), the cultivar K-7 resulting from a selection work in Kenya, 11 samples representing the different colleting sites of the ORSTOM mission in Ethiopia, and two accessions collected in Kenya, were included in Lashermes P. etc′s study. Their study indicated a relatively large genetic diversity within the Arabica germplasm collection and demonstrated the importance of collecting missions. As expected from their origin, they were not able to distinguished the cultivars belonging to the same type, either bourbon or typical. On the other hand, both bourbon and typical types showed important difference. The cultivar K7, which has been grown on a large scale in Kenya, appeared closely related to one of the accessions collected in the north of Kenya.

Chaparro A P, etc[6] evaluated the genetic diversity of 50 wild and semi-wild accessions of the *Coffea arabica* L. germplasm collection, gathered by the FAO and ORSTOM missions to Ethiopia, and maintained in Colombia by CENICAFE with RAPD markers. Among the subset of 9 accessions evaluated for RAPD markers, a total of 24 primers produced polymorphism (57% of the primers tested). A total of 401 fragments were amplified for an average of 9,5-fragments per primer and 137 of those fragments were founed polymorphic(34. 2%). Six combinations of 5 primers were constructed from the 24 primers that generated polymorphic bands. There was no association between the grouping and the origin site in Ethiopia in their study. It is also must be noted that 60. 7% of the accessions were collected from this region. Nevertheless, this observation agrees with the belief that the Kaffa region

is the origin site of C. *arabica* and hence where the highrest genetic variability of the species is expected to be found. For breeding purposes, our results indicate that a larger potential than previously reported is present in the germplasm collection of *C. arabica* from Ethiopia, which can be used to improve popular commercial varieties such as Caturra.

3 Other molecule marker technology applicaton in coffee breeding program

Lashermes P, etc[7] used restriction fragment length polymorphism (RFLP) markers in combination with genomic in situ hybridisation (GISH) to investigate the origin of the allotetraploid species *Coffea arabica* (2n = 44). By comparing the RFLP patterns of potential diploid progenitor species with those of *C. arabica*, the sources of the two sets of chromosomes, or genomes, combined in *C. arabica* were identified. The genome organization of *C. arabica* was confirmed by GISH using simultaneously labelled total genomic DNA from the two putative genome donor species as probes. These results clearly suggest that *C. arabica* is an amphidiploid formed by hybridization between *C. eugenioides* and *C. canephora*, or ecotypes related to these diploid species. Our results also indicate low divergence between the two constituent genomes of *C. arabica* and those of its progenitor species, suggesting that the speciation of *C. arabica* took place relatively recently. Precise localization in Central Africa of the site of the speciation of *C. arabica*, based on the present distribution of the coffee species, appears difficult, since the constitution and extent of tropical forest has varied considerably during the late Quaternary period.

Milene Silvestrini, etc[8] assessed the genetic diversity among 115 coffee accessions from the Coffea Germplasm Collection of IAC using SSR markers. The germplasm represents 73 accessions of *Coffea arabica* derived from sporrtaneous and subspontaneous plants in Ethiopia and Eritrea, species center of origin and diversity, 13 commercial cultivars of *C. arabica* developed by the Breeding Program of IAC, 1 accession of *C. arabica* cv. Geisha′, 13 accessions of *C. arabica* from Yemen, 5 accessions of *C. eugenioides*, 4 accessions of *C. racemosa* and 6 accessions of *C. canephora*. Genetic analysis was performed using average number of alleles per locus (A), proportion of polymorphic loci (P), Shannon′s genetic index (H′ and G′ST) and clustering analysis. All evaluated species were distinguished by a cluster analysis based on Jaccard′s coefficient. Differentiation between the cultivated plants of *C. arabica* and accessions derived from spontaneous and subspontaneous plants was observed. Spontaneous and subspontaneous accessions from Ethiopia were separated according to the geographical origin: east and west of the Great Rift Valley. Cultivated plants showed a low genetic diversity with a division in two groups: accessions from Yemen and Brazilian commercial cultivars. The results agreed with previously reported narrow genetic basis of cultivated plants of *C. arabica* and supported the hypotheses about domestication of the species. Their study also showed a significant genetic diversity among accessions from Ethiopia and Eritrea present in the Germplasm Collection of IAC. The diversity is specially observed in accessions from Sidamo, Kaffa and Illubabor indicating their importance as source of genetic variability for coffee breeding programs[9].

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