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ISSR 指纹图谱分析鉴定油茶遗传多样性的研究进展

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摘　要：油茶是一种具有较高经济价值的植物，在我国主要分布于西南地区。但长期以来，对我国油茶植物的品种资源尚未清楚，本文对 ISSR 分子标记鉴定油茶重要栽培群体遗传多样性和亲缘关系进行了分析，探讨了遗传多样性和亲缘关系的信息将有助于建立一个核心种质库和开发具有较高经济价值的油茶新品种。

关键词：遗传多样性；指纹图谱；油茶

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Research Advances on ISSR Repeat Fingerprints Analysis to Assess Genetic Diversity in *Camellia oleifera*

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Abstract：*Camellia oleifera* is an important economic crop which is mainly grown in southwest China. However，little is known about the germplasm resources of *C. oleifera* in China. In this study，the genetic diversity of some *C. oleifera* varieties was identified and their phylogenetic relationship was further analyzed using ISSR marker. Thus，these findings served as valuable information for the establishment of core collection as well as the breeding of new varieties with high economic value.

Key words：genetic diversity；fingerprints；*Camellia oleifera*

1　Fatty acids composition in seed oil

The oiltea camellia（*Camellia oleifera* Abel）is a traditional woody vegetable oil plant，which is mainly distributed in southern part of China[1]. Currently，there are about 400×10^4 hm^2 of *C. oleifera* producing around 560×10^4 tons of nuts annually. The pipes of nuts are usually processed in oil-mills，and more than 300×10^4 tons of shell as agricultural waste are discarded or burnt rather than effectively reused[2-3]. Furthermore，seeds of oil tea have been utilized in China for more than 2000 years[4]，which contain tea saponin and tea oil. The defatted seed residues，called seed cakes or seed meals，are always treated as detergent or

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organic fertilizer without high economic value. At present, it is difficult to extract high quality tea saponin from tea-cakes of *C. oleifera* seeds. Tea oil is the main cooking oil in southern provinces of China, especially in Hunan and Jiangxi. The high nutrition value of tea oil is mainly due to its high oleic acid content, which constitutes up to 88% of the fatty acids[5]. Tea oil's flavor is comparable to olive oil. According to Lee[6], it is abundant in unsaturated fatty acids, and has been shown to have hepato-protective and antioxidant proper ties. The fruit hull of oiltea camellia is often discarded because of undeveloped economic benefit. Recently, polyphenols were identified as one group of the beneficial components in oiltea camellia[7]

Fatty acid synthase is a lipogenic enzyme that participates in de novo synthesis of long-chain fatty acids. FAS is down regulated in most normal human tissues. In contrast, it is often highly expressed in human cancers, including carcinoma of the colon, prostate, ovary, and breast. The differential distribution in tissue renders inhibition of FAS a potential therapeutic approach in cancer treatment[8]. Loftus[9] found that mice treated with C75 could reduce body weight by affecting both food intake and energy expenditure. Orlistat, a drug widely used for weight control in obese patients, inhibited tumor growth through inhibiting FAS[10]. FAS inhibitors are receiving more attention because increasing clinical and experimental evidence suggests that FAS is a potential therapy target of both obesity and cancer.

2　Genetic diversity of populations

Genetic diversity is very important in plant breeding programs[11]. Knowledge of the genetic relationships between different accessions can greatly aid the development of efficient germplasm management and utilization strategies[12]. Morphological trait measurement is one of the methods that has been widely employed to estimate the genetic diversity of species. They are commonly used parameters since they provide a simple technique of quantifying genetic variation while simultaneously assessing genotype performance in relevant growing environments[13]. However, assessing morphological traits is labour-intensive, and the phenotypic plasticity of plants makes environmental variation a major problem[14]. As the wild resources decreased rapidly, *C. oleifera* was extensively cultivated since 1970s. At present commercial oil tea mainly depends on cultivated resource with its planting area concentrated in China's southern provinces. Some area of these provinces had become the main production area of oil tea. The species predominantly propagate sexually by seeds at most plant regions. In rural area, farmers usually collected all mature seeds directly from local wild resources randomly, mixed them together, and planted them in the fields[5]. However, is it likely that the cultivation practices like oil tea result in the homogeneity or decrease of genetic diversity after several decades of cultivation? This question is becoming increasingly severe attended by rapid decrease of wild gene pools of this species and lack of good cultivars.

2.1　Genetic diversity of plants revealed by ISSR markers

The inter-simple sequence repeat (ISSR) marker system is a polymerase chain reaction (PCR)-based technique that uses a single amplification primer composed of a microsatellite motif to target a subset of simple sequence repeats (SSRs) or microsatellites[15]. SSRs, or microsatellites, and ISSRs have been recognized as useful molecular markers in marker-assisted selection, the analysis of genetic diversity, population genetic analysis, and other purposes in various species[16].

2.2　Optimization of ISSR reaction system in plant

RAPD and SSR markers exhibited different levels of polymorphism. Similar results have been obtained in wheat, corn and rice. Previously, studies about the genetic diversity of *C. oleifera* have been undertaken using different molecular markers. Although previous researches have provided preliminary data regarding the genetic diversity at species level, more works need to be done about the genetic diversity and population structure within cultivated populations in main producing area of China. A number of molecular markers, including restriction fragment length polymor-

phism (RFLP), random-amplified polymorphic DNA(RAPD), arbitrary primed polymerase chain reaction(AP-PCR), DNA amplified fingerprinting (DAF), simple (short) sequence repeat (SSR), short tandem repeat(STR), sequence characterized amplified region (SCAR), sequence-tagged sites (STSs), amplified fragment length polymorphism (AFLP), inter simple sequence repeat(ISSR), expressed sequence tag(EST)-PCR and cleaved amplified polymorphic sequences(CAPS) derived from EST-PCR markers are available for genetic analysis of tissue culture-raised plants. Although reviews of these techniques are plentiful[17] due to the rapidity with which relevant technology is proceeding, these may not remain effective for long. The development of PCR has set the stage to overcome many of the shortfalls in the Southern blotting RFLP technique[18]. PCR-based DNA marker systems can be divided into two basic classes: those that use primers designed from arbitrary or non-specific sequences such as RAPD and AFLP, and those that use primers designed from known sequence for targeting a single specific locus such as SSRs and STSs. Thus, in order to evaluate genetic diversity at populations level among wild plant species, the simple, reliable, and cheap molecular markers should be developed firstly. Saiki[19] indicated that the ISSR analysis needs no prior DNA sequence information with low costs of development and easy laboratory procedures. Therefore this technology has been widely used to investigate genetic diversity and population genetic structure of the wild plants[14, 20]. In addition, the ISSRs were applied to the study of SSR distribution and frequency in whole genomes of plants [21]. ISSR markers are more reliable than RAPD(random amplification polymorphic DNA) makers because of longer length of the primers and higher annealing temperature than that of RAPD markers. The credible and reproducible assay using ISSR technique has been conducted in the genetic diversity analysis of many plants [22-25].

3 Conclusions

The introduction of DNA-based markers allows direct comparisons of different genetic material, independent of environmental influences. The degree of similarity between banding patterns can provide information about genetic similarity and relationships between the samples studied. Each marker system has its own strengths and limitations, making the choice of marker an important decision. In order to improve its genetic diversity, new genetic resources should be introduced from its wild species or from other cultivated populations. Some studies provided evidence that the ISSR procedure is an informative and suitable approach to the examination of the molecular polymorphism and the phylogenic relationships in the fig germplasm. Work is currently in progress to enlarge the number of markers by the use of other molecular technologies in order to have a deeper insight into the molecular polymorphisms and to establish a varietal identification key in this plant. Such practices might be an effective way for the maintenance and conservation of gene pools of oil tea plants.

The oil tea species are invaluable gene pool for germplasms to develop novel cultivars of pasture grasses and medicinal plants. To date, little study has been done on genetic assessment of Chinese *C. oleifera* species using the molecular markers. A systematic genetic assessment of plant resources will help to find the relationship between the economic traits and molecular markers. At the same time, the genetic analysis with molecular makers is promotive to the study of genetic polymorphism of wild resources. Furthermore, genetic studies of plant resources has substantially decreased the redundancy of germplasm conservation and facilitated the construction of a core germplasm collection, which is important for efficient use of gene resources in plant breeding.

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