Establishment of SCoT Molecular Marker System for Rehmannia and Postprint of Fingerprinting

Authors: Yang Ke, Zhou Yanqing, Duan Hongying, Guo Mengmeng

Date: 2018-12-04T00:00:00+00:00

Abstract

Start Codon Targeted Polymorphism (SCoT) marker is a novel target gene molecular marker based on single-primer amplification reaction. This study employed both L25(5^6) orthogonal design and single-factor methods to optimize five factors affecting Rehmannia SCoT-PCR reaction (template DNA concentration, primer concentration, ddH2O and Mix amounts, and annealing temperature). The optimized reaction system was: total volume of 25 L, containing 8 L ddH2O, 1 L template DNA (80 ng· L^-1), L primer (8 mol·L^-1) and 15 L Mix, with an annealing temperature of 45°C. Using 30 Rehmannia germplasm materials, the optimized SCoT-PCR system was repeatedly validated, obtaining amplification profiles with rich polymorphism and clear bands, proving that this reaction system is stable and reliable. Using this system, 32 SCoT primers were screened twice, yielding 14 primers with clear amplification products, good repeatability, and relatively high numbers of polymorphic bands. Finally, SCoT fingerprinting profiles for the above-mentioned 30 germplasm samples from two Rehmannia species were constructed using five primers including SCoT4. These five SCoT primer fingerprinting profiles could distinguish seven commonly used Rehmannia cultivated varieties. The study demonstrates that the SCoT molecular marker system is suitable for research on genetic relationships and genetic diversity of main Rehmannia varieties, and the constructed fingerprinting profiles also provide a reference basis for distinguishing the seven common Rehmannia cultivated varieties. These results lay a foundation for research on germplasm resource genetic relationships, molecular breeding, and genetic diversity in Rehmannia.

Full Text

Preamble

DOI: 10.11931/guihaia.gxzw201808008

Title: Establishment of SCoT Molecular Marker System and Construction of Fingerprint for *Rehmannia glutinosa*

Authors: YANG Ke[1], ZHOU Yanqing[1,2,3*], DUAN Hongying[1], GUO Mengmeng[1]

Affiliations: 1. College of Life Sciences, Henan Normal University, Xinxiang 453007, Henan, China 2. Key Laboratory for Microorganisms and Functional Molecules (Cultivation Base), Henan Normal University, Xinxiang 453007, Henan, China 3. Engineering Technology Research Center of Nursing and Utilization of Genuine Chinese Crude Drugs, Henan Normal University, Xinxiang 453007, Henan, China

---

Abstract

Start codon targeted polymorphism (SCoT) markers represent a novel type of target gene molecular marker based on single-primer amplification reactions. This study employed both L25(5) orthogonal design and single-factor methods to optimize five key factors affecting SCoT-PCR reactions in *Rehmannia glutinosa*: template DNA concentration, primer concentration, ddH O volume, Mix quantity, and annealing temperature. The optimized reaction system consisted of a total volume of 25 L containing 8 L ddH O, 1 L template DNA (80 ng · L ^{1}), 1 L primer (8 mol · L ^{1}), and 15 L Mix, with an annealing temperature of 45 °C. Using 30 *Rehmannia* germplasm materials, the optimized SCoT-PCR orthogonal system was repeatedly validated, yielding amplification profiles with rich polymorphism and clear bands that demonstrated the system' s stability and reliability. Subsequently, 32 SCoT primers were screened twice using this system, resulting in 14 primers that produced clear, reproducible amplification products with relatively high polymorphic bands. Finally, SCoT fingerprints were constructed for 30 germplasm accessions across two *Rehmannia* species using five primers including SCoT4. These five SCoT primer fingerprints successfully distinguished seven commonly cultivated *Rehmannia glutinosa* varieties. The results indicate that the SCoT molecular marker system is suitable for studying genetic relationships and diversity among major *Rehmannia* varieties, and the constructed fingerprints provide a reference for distinguishing seven common cultivated varieties. These findings establish a foundation for research on germplasm relationships, molecular breeding, and genetic diversity in *Rehmannia*.

Keywords: Start codon targeted polymorphism (SCoT); single-factor test; orthogonal design; primer screening; variety identification

Funding: This work was supported by the National NSFC-Henan Talent Training Joint Fund (U1304304), Henan Provincial Department of Education Science and Technology Research Key Project (14B180028), Henan Normal University 2016 National Project Cultivation Fund Project (2016PL18), and College Students Innovation and Entrepreneurship Training Program Project (0424, 0438).

Author Information: YANG Ke (1994–), female, from Nanyang, Henan, Master' s student, research direction: microbial genetics, (E-mail) ykelucky@163.com.

Corresponding Author: ZHOU Yanqing, Ph.D., Professor, mainly engaged in genetics research, (E-mail) yqzhou@htu.cn.

Introduction

Rehmannia glutinosa is a perennial herb belonging to the family Scrophulariaceae and genus *Rehmannia*, which includes six species: *R. chingii*, *R. piasezkii*, *R. elata*, *R. henryi*, *R. solanifolia*, and *R. glutinosa*, distributed across China, Japan, and Korea. In China, *R. glutinosa* is primarily found in Henan, Shanxi, Liaoning, Shandong, Shaanxi, and Anhui provinces. The roots of *R. glutinosa* hold significant economic value as both medicinal material and food, attracting considerable research attention.

Regarding DNA molecular marker studies in *R. glutinosa*, various markers have been applied for genetic diversity evaluation, classification, identification, fingerprinting, population genetics, and diagnostic studies, including RAPD, ISSR, SRAP, SCAR, ITS, AFLP, chloroplast DNA non-coding regions, and EST-SSR markers (Guo Guanying, 2013; Zhou Yanqing et al., 2015; Wang Wanshen, 2016; Xia Zhi et al., 2016; Feng Fajie et al., 2015; Liu et al., 2015). However, these markers each have limitations: RAPD suffers from instability, AFLP is time-consuming and expensive, and SSR markers are difficult to develop. Therefore, new molecular markers for *R. glutinosa* are needed.

Start codon targeted polymorphism (SCoT) is a novel DNA molecular marker technology based on single-primer amplification reactions. It employs single primers designed from conserved regions flanking the ATG start codon in plant genes to amplify genomic regions on both sides of ATG, generating dominant polymorphic markers biased toward candidate functional gene regions. SCoT offers several advantages: it can obtain trait-linked target genes, track traits, is simple to operate, highly reproducible, exhibits rich polymorphism, and has good primer universality. The technology has been widely applied in genetic diversity analysis of durum wheat (Guo et al., 2016), phylogenetic relationships between intermediate wheatgrass and common wheat (Xu Lintao, 2015), identification of Tunisian citrus species (Mahjbi et al., 2015), classification of *Diospyros lotus* (Yang et al., 2015), fingerprint construction (Luo Ting et al., 2013), genetic homozygosity assessment of regenerated *Helicteres isora* plants

(Mariappan et al., 2016), and molecular genetic linkage map construction (Long Zhijian et al., 2015).

Additionally, due to long-term vegetative propagation and inter-regional introduction, most *R. glutinosa* varieties have degenerated after years of cultivation, with severely reduced quality and yield and inconsistent external morphology. However, current research on *R. glutinosa* has primarily focused on breeding, chemistry, and pest control (Liu Chengwei et al., 2007; Ji Wei et al., 2008; Chen Daxia et al., 2009, 2011). To date, no reports have applied SCoT marker technology to *R. glutinosa*, and molecular-level studies on its genetic diversity and phylogenetic relationships remain relatively scarce (Chen Daxia et al., 2012). Therefore, this study aimed to develop new molecular markers for *R. glutinosa*, optimize the SCoT-PCR system, screen appropriate primers, and construct SCoT fingerprints for 30 germplasm accessions. This work establishes a foundation for genetic diversity analysis and variety identification in *R. glutinosa* and provides a reference for distinguishing seven common cultivated varieties: Hongshuwang, Kangyu 831, Beijing No. 3, Beijing No. 2, Jinzhuangyuan, Jinjiu, and Wen 85-5.

1.1 Experimental Materials

Rehmannia germplasm was collected from four cities/counties in Henan, two districts/counties in Shandong, and East China Normal University in Shanghai, comprising two species (*R. piasezkii* and *R. glutinosa*) with a total of 30 accessions. In May 2016, graduate student Wang Wanshen collected fresh young leaves from each location (three plants per site as replicates), which were rapidly dried with silica gel and stored at -70 °C. All materials were identified by Professor Duan Hongying of Henan Normal University and confirmed as *R. piasezkii* or *R. glutinosa* germplasm through ITS sequencing and NCBI BLAST comparison by graduate student Wang Wanshen (Wang Wanshen, 2016) (Table 1). The Taq MasterMix used in experiments was synthesized by Beijing CoWin Biotech Co., Ltd., offering advantages of good stability, high sensitivity, simplicity, and strong specificity. Thirty-two single primer sequences were selected from literature (Chen Daxia et al., 2012; Li Pirui et al., 2013; Jiang Yaqin et al., 2014) and synthesized by Beijing Sunbiotech Co., Ltd., designated as SCoT1–SCoT32.

1.2 DNA Extraction and Detection

Fresh *Rehmannia* leaves were used for DNA extraction via a modified CTAB method. DNA integrity was assessed using 1% agarose gel electrophoresis, and concentration and purity were measured with a UV spectrophotometer. Samples were diluted to the required concentration and stored at -20 °C for subsequent use.

1.3 Establishment of Basic SCoT-PCR System and Target Primer Screening

Based on literature (Luo Ting et al., 2013; Xu Lintao, 2015; Guo et al., 2016; Mahjbi et al., 2015), a basic SCoT-PCR reaction system was initially established for *R. glutinosa* to screen 32 primers. The SCoT-PCR reaction system (20 L) contained 8 L ddH O, 1 L template DNA (80 ng· L ^{1}), 1 L primer (8 mol·L ^{1}), and 10 L Mix. The amplification program consisted of: initial denaturation at 94 °C for 4 min; 36 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 2 min; final extension at 72 °C for 5 min; and storage at 4 °C. PCR products were detected via 1% agarose gel electrophoresis and photographed for analysis.

1.4 Orthogonal Experimental Design for SCoT-PCR

Wen 85-5 is the most common cultivated variety of *R. glutinosa*. DNA from this variety was selected as template, and SCoT15 (screened in Section 1.3) was used as primer. Five factors—template DNA concentration, primer concentration, ddH O volume, Mix quantity, and annealing temperature—were evaluated at five levels each (Table 2) using L25(5) orthogonal experimental design to optimize the basic SCoT-PCR system, with two replicates. PCR amplification was performed on a Tgradient thermocycler with the following program: initial denaturation at 94 °C for 4 min; 36 cycles of denaturation at 94 °C for 1 min, annealing at temperatures specified in the orthogonal table for 1 min, and extension at 72 °C for 2 min; and storage at 4 °C. After amplification, 8–10 L of product was subjected to 1% agarose gel electrophoresis in 1×TAE buffer and photographed using a Gel Doc™ EZ imager.

1.5 Annealing Temperature Optimization

Annealing temperature is a critical factor affecting PCR specificity. Based on the optimal system identified from orthogonal experiments, a single-factor experiment was designed for further gradient optimization of annealing temperature. Five temperature gradients were tested: 45.0 °C, 47.0 °C, 51.3 °C, 53.3 °C, and 55.0 °C. The reaction program was identical to the orthogonal system except for annealing temperature.

1.6 Verification of Optimal Orthogonal System

The 14 primers screened in Section 1.3 and the optimized orthogonal system from Section 1.5 were used to amplify DNA templates from 30 *Rehmannia* germplasm accessions. Amplification products were detected via 1% agarose gel electrophoresis to verify the stability, universality, and reproducibility of the system.

1.7 Data Processing

Bands were manually scored based on their relative positions on the gel electropherogram, ordered from largest to smallest molecular weight. Clear and identifiable bands were recorded as "1," absent bands as "0," and missing data as "9." Unclear bands were excluded from recording. A digital SCoT fingerprint table was established for the 30 *Rehmannia* germplasm accessions.

2.1 Quality and Concentration Detection of *Rehmannia* DNA

DNA integrity was assessed via 1% agarose gel electrophoresis, which revealed clear, intact bands without smearing or protein contamination. DNA concentration and purity were measured using a microspectrophotometer, with OD260/OD280 ratios ranging from 1.8 to 2.0, indicating high purity without contaminant interference and suitability for PCR amplification.

2.2 Establishment of Basic SCoT-PCR System and Primer Screening

Using the basic system and amplification program, DNA templates from common cultivated varieties including Wen 85-5, Jinjiu, Jinzhuangyuan, and Hongshuwang were used to screen 32 SCoT primers via SCoT-PCR (Figure 1 [Figure 1: see original paper]). Fourteen primers producing clear amplification products with good repeatability and relatively high polymorphic bands were selected for formal PCR amplification (Table 3).

2.3 Verification of Orthogonal Experiments

Amplification results from the orthogonal experiments showed that 18 of the 25 treatment combinations produced bands, while treatments 10, 14, 16, 18, 20, 22, and 23 yielded no products. Treatments 5, 6, 7, and 19 produced minimal amplification, whereas treatments 8 and 9 generated clear, easily identifiable polymorphic banding patterns (Figure 3 [Figure 3: see original paper]). Based on comprehensive visual analysis of all bands, the optimal SCoT-PCR reaction system for *R. glutinosa* was determined to be: 8 L ddH O, 80 ng · L ^{1} template DNA, 8 mol · L ^{1} primer, 15 L Mix, and an annealing temperature of 45 °C, in a total volume of 25 L.

2.4 Annealing Temperature Optimization

Among the five annealing temperatures tested (Figure 4 [Figure 4: see original paper]), amplification at 45.0 °C produced strong band intensity with high specificity and easy identification. Temperatures of 47.0 °C and 55.0 °C yielded fewer bands with weaker background. At 51.3 °C and 53.3 °C, band number increased with rising temperature and background intensified, but background

weakened again at 55.0 °C. Therefore, based on clear band amplification and polymorphism, the optimal annealing temperature was determined to be 45.0 °C.

2.5 Verification of Optimal *Rehmannia* Reaction System

Using the 14 initially screened primers and the optimized orthogonal system, SCoT-PCR analysis was performed on DNA templates from 30 *Rehmannia* germplasm accessions, with products detected via agarose gel electrophoresis. Except for SCoT21 and SCoT25, all primers amplified clear, moderately bright, highly polymorphic bands from the test materials. For example, SCoT15 and SCoT27 produced clear, easily identifiable polymorphic profiles across the 30 *Rehmannia* materials (Figure 5 [Figure 5: see original paper], Figure 6 [Figure 6: see original paper]), confirming that the established and optimized SCoT molecular marker system is suitable for subsequent genetic diversity studies of *R. glutinosa* genomic DNA.

2.6 SCoT Primer Amplification Analysis

Through two rounds of screening of 32 SCoT primers, 14 primers producing clear, reproducible amplification products with relatively high polymorphic bands were obtained. Amplification analysis of 30 *Rehmannia* germplasm accessions using these 14 primers revealed: (1) a total of 103 loci were amplified, of which 96 were polymorphic, yielding a polymorphism rate of 94.2%; (2) genetic similarity coefficients among the 31 materials ranged from 0.4719 to 0.9223, with an average of 0.70, indicating that SCoT markers can reveal high genetic diversity among varieties. Five primers were ultimately selected to construct fingerprints for the 30 *Rehmannia* germplasm accessions, successfully distinguishing seven commonly cultivated *R. glutinosa* varieties.

2.7 Fingerprint Construction

Based on amplification results from five primers (SCoT4, SCoT5, SCoT11, SCoT15, and SCoT27), clear and identifiable bands on electropherograms were scored as "1," absent bands as "0," and missing data as "9." A digital SCoT fingerprint table was established for the 30 *Rehmannia* germplasm accessions (Table 4). These five polymorphic SCoT primers effectively distinguished seven commonly cultivated *R. glutinosa* varieties: SCoT4 produced specific bands to differentiate Beijing No. 2, Jinzhuangyuan, and Jinjiu; SCoT27 distinguished Hongshuwang and Kangyu 831; SCoT5 amplified specific bands for Beijing No. 3; SCoT11 generated specific bands for Wen 85-5; and SCoT27 amplified specific bands for Jinjiu.

3 Discussion

Traditional molecular markers such as RAPD, ISSR, EST-SSR, SCAR, and SRAP have proven effective and reliable but each has distinct characteristics and limitations (Long Zhijian et al., 2015), including high cost, poor experimental repeatability, and instability. SCoT markers, as a novel DNA molecular marker technology using 18-bp single primers, offer advantages including low DNA quality requirements, minimal DNA quantity needs, no requirement for known sequence information for primer design, low primer mismatch rates, good primer universality, and high amplification product polymorphism. Additionally, detection via agarose gel electrophoresis is simple, inexpensive, and rapid. In recent years, SCoT has been applied to various crops including watermelon (Yang Jing et al., 2016), peanut (Xiong Faqian et al., 2010), loofah (Jiang Yaqin et al., 2014), carambola (Ou Jingli et al., 2015), and sugarcane (Chen Pinghua et al., 2011), as well as in plant genetic diversity analysis (Guo et al., 2016), phylogenetic studies (Xu Lintao, 2015), species identification (Mahjbi et al., 2015), classification (Yang et al., 2015), fingerprint construction (Luo Ting et al., 2013), genetic homozygosity assessment of regenerated plants (Mariappan et al., 2016), and molecular genetic linkage map construction (Long Zhijian et al., 2015).

However, SCoT technology has rarely been applied to *R. glutinosa* genetic diversity analysis, with only Chen Daxia' s study on genetic variation and differentiation in *Scrophularia*. Existing molecular markers for *R. glutinosa* are limited to SRAP, SCAR, RAPD, ISSR, ITS, AFLP, species identification (Mahjbi et al., 2015), chloroplast DNA non-coding regions (rbcL, ndhF, rps16, trnL-F, psbA-trnH, and matK), and EST-SSR markers published by Feng Fajie (2015), Guo Guanying (2013), and Cheng Yueqin (2013). Therefore, this study utilized SCoT molecular markers to analyze genetic polymorphism in 30 *Rehmannia* accessions, providing a new molecular marker technology for *R. glutinosa* and other species and offering a basis for *Rehmannia* germplasm identification. The 14 SCoT primers screened in this study exhibited high polymorphism across 30 *Rehmannia* resources, with an average polymorphism rate of 94.2%, which is higher than reported in some other studies: Li Pirui et al. (2013) reported 90.4% average polymorphism using 12 SCoT primers in 18 chrysanthemum materials; Chen Hu et al. (2010) reported 85.8% average polymorphism using 24 primers in 24 longan varieties; and Jiang Yaqin et al. (2014) reported 87.57% polymorphism using 10 primers in 81 loofah varieties. These primers amplified specific bands in some cultivated varieties, though whether these specific bands are linked to particular traits requires further investigation.

This study aimed to develop new molecular markers for *R. glutinosa* and overcome limitations of SCoT-PCR technology. The optimized SCoT-PCR reaction system for *R. glutinosa* was established as: total volume 25 L containing ddH O 8 L, template DNA 80 ng· L ^{1}, primer 8 mol·L ^{1}, Mix 15 L, and annealing temperature 45 °C. This SCoT-PCR system was validated for universality and stability across *R. glutinosa* cultivars, two different *Rehmannia* species, landraces,

and wild species, all yielding richly polymorphic profiles. The system is suitable for fingerprint construction, identification, and genetic diversity analysis of *Rehmannia* germplasm, and establishes a foundation for subsequent research including SCAR marker development.

References

CHEN DX, LI LY, PENG R, et al., 2009. SRAP study on genetic relationship and genetic diversity of three cultivation types of *Scrophularia sinensis* [J]. *Chin J Mat Med*, 34(2):138-142. [陈大霞, 李隆云, 彭锐, 等, 2009. 玄参 3 种栽培类型遗传关系和遗传多样性的 SRAP 研究 [J]. 中国中药杂志, 34(2):138-142.]

CHEN DX, LI LY, SONG CQ, et al., 2011. Several drugs to control the efficacy of Xuanxuan spot blotch [J]. *Anhui Agric Sci*, 22(15):13340-13341. [陈大霞, 李隆云, 宋春泉, 等, 2011. 几种药剂防治玄参斑枯病药效试验 [J]. 安徽农业科学, 22(15):13340-13341.]

CHEN DX, ZHANG X, WANG Y, et al., 2012. Analysis of genetic diversity of *Scrophularia* germplasm resources by SCoT markers [J]. *Chin J Mat Med*, 37(16):2368-2372. [陈大霞, 张雪, 王钰, 等, 2012. 应用 SCoT 标记分析玄参种质资源的遗传多样性 [J]. 中国中药杂志, 37(16):2368-2372.]

CHEN H, HE XH, LUO C, et al., 2010. Genetic diversity analysis of SCoT in 24 varieties of Longyan [J]. *J Hortic*, 37(10):1651-1654. [陈虎, 何新华, 罗聪, 等, 2010. 龙眼 24 个品种的 SCoT 遗传多样性分析 [J]. 园艺学报, 37(10):1651-1654.]

CHEN PH, CHEN RK, XU LP, et al., 2011. Whole genome amplification of sugarcane single pollen (WGA) and SCoT molecular markers [J]. *J Trop Crops*, 32(11):2069-2075. [陈平华, 陈如凯, 许莉萍, 等, 2011. 甘蔗单花粉全基因组扩增 (WGA) 与 SCoT 分子标记研究 [J]. 热带作物学报, 32(11):2069-2075.]

CHENG YQ, JIAO ZB, ZHANG P, et al., 2013. Construction and characterization of microsatellite enriched library of *Rehmannia glutinosa* [J]. *Seed*, 32(5):12-16. [程月琴, 焦振彬, 张佩, 等, 2013. 地黄微卫星富集文库构建及特性分析 [J]. 种子, 32(5):12-16.]

FENG FJ, LI MJ, GU L, et al., 2015. Development of EST-SSR markers and establishment of amplification system for *Rehmannia glutinosa* [J]. *Guangdong Agric Sci*, 42(10):120-126. [冯法节, 李明杰, 古力, 等, 2015. 地黄 EST-SSR 标记的开发及扩增体系的建立 [J]. 广东农业科学, 42(10):120-126.]

GUO GY, 2013. Construction of large-capacity transcriptome library of *Rehmannia glutinosa* and development and identification of EST-SSR marker [D]. Zhengzhou: Henan Agricultural University. [郭冠瑛, 2013. 地黄大容量转录组文库的构建及 EST-SSR 标记的开发与鉴定 [D]. 郑州: 河南农业大学.]

GUO J, YU X, YIN H, et al., 2016. Phylogenetic relationships of *Thinopyrum* and *Triticum* species revealed by SCoT and CDDP markers [J]. *Plant Syst Evol*, 302(9):1301-1309.

JI W, LIANG ZS, JIANG ZM, et al., 2008. Study on optimization formula

fertilization technology of high-yield cultivation of *Scrophularia sinensis* [J]. *J NW A & F Univ (Nat Sci Ed)*, 36(2):170-174. [纪薇, 梁宗锁, 姜在民, 等, 2008. 玄参高产栽培优化配方施肥技术研究 [J]. 西北农林科技大学学报 (自然科学版), 36(2):170-174.]

JIANG YQ, LI Y, LI WJ, et al., 2014. Application of SCoT molecular marker technology in loofah [J]. *J S Agric Sci*, 45(12):2117-2122. [蒋雅琴, 黎炎, 李文嘉, 等, 2014. SCoT 分子标记技术在丝瓜上的应用 [J]. 南方农业学报, 45(12):2117-2122.]

LIU CW, BI ZM, ZHU YF, et al., 2007. HPLC quantitative analysis of four main active components in *Scrophulariae* [J]. *Chin J Mat Med*, 42(21):1614-1616. [刘承伟, 毕志明, 祝艳斐, 等, 2007. 玄参中 4 种主要活性成分的 HPLC 定量分析 [J]. 中国药学杂志, 42(21):1614-1616.]

LI PR, JIANG JF, CHEN SM, et al., 2013. Application of SCoT molecular marker technology in genetic diversity analysis of *Chrysanthemum* [J]. *J Hortic*, 40(10):2015-2025. [李丕睿, 蒋甲福, 陈素梅, 等, 2013. 菊属植物 SCoT 分子标记技术在遗传多样性分析中的应用 [J]. 园艺学报, 40(10):2015-2025.]

LONG ZJ, FAN LZ, XU G, et al., 2015. Progress in the application of SCoT molecular markers in plant research [J]. *J Plant Gene Resour*, 16(2):336-343. [龙治坚, 范理璋, 徐刚, 等, 2015. SCoT 分子标记在植物研究中的应用进展 [J]. 植物遗传资源学报, 16(2):336-343.]

LUO T, YANG HX, QIN HF, et al., 2013. Application of SCoT molecular markers in construction of cut-to-family genetic maps [J]. *J Plant Gene Resour*, 14(4):704-710. [罗霆, 杨海霞, 岑华飞, 等, 2013. SCoT 分子标记在割手密遗传图谱构建中的应用 [J]. 植物遗传资源学报, 14(4):704-710.]

MAHJBI A, BARAKET G, OUESLATI A, et al., 2015. Start codon targeted (SCoT) markers provide new insights into the genetic diversity analysis and characterization of Tunisian Citrus species [J]. *Biochem Syst Ecol*, 61:390-398.

MARIAPPAN M, THIRUPPATHI SK, MANDALI VR, 2016. Organogenesis and evaluation of genetic homogeneity through SCoT and ISSR markers in *Helicteres isora* L., a medicinally important tree [J]. *S Afr J Bot*, 106:204-210.

OU JL, XUN JF, YU BN, et al., 2015. Establishment and verification of SCoT labeled PCR reaction system in carambola [J]. *S Chin Fruit Tree*, 44(3):16-21. [欧景莉, 覃剑峰, 余炳宁, 等, 2015. 杨桃 SCoT 标记 PCR 反应体系建立与验证 [J]. 中国南方果树, 44(3):16-21.]

WANG WS, 2016. Screening and identification of DNA barcode sequences of *Rehmannia glutinosa* [D]. Xinxiang: Henan Normal University. [王婉珅, 2016. 地黄 DNA 条形码序列的筛选与鉴定研究 [D]. 新乡: 河南师范大学.]

XIONG FQ, JIANG J, ZHONG RC, et al., 2010. Application of target codon polymorphism (SCoT) molecular marker technique in peanut [J]. *Acta Agronom Sin*, 36(12):2055-2061. [熊发前, 蒋菁, 钟瑞春, 等, 2010. 目标起始密码子多态性 (SCoT) 分子标记技术在花生属中的应用 [J]. 作物学报, 36(12):2055-2061.]

XU LT, 2015. Identification of wheat-intermediate ryegrass germplasm line [D]. Tai' an: Shandong Agricultural University. [徐林涛, 2015. 小麦—中间偃麦草种质系的鉴定 [D]. 泰安: 山东农业大学.]

XIA Z, WANG LJ, HUANG Y, et al., 2016. Identification of DNA barcodes of *Rehmannia* and the origin of *Rehmannia* cultivation [J]. *Chin Trad Herb Drugs*, 47(4):648-654. [夏至, 王璐静, 黄勇, 等, 2016. 地黄属植物 DNA 条形码鉴定及地黄栽培起源研究 [J]. 中草药, 47(4):648-654.]

YANG Y, YANG TT, JING ZB, 2015. Genetic diversity and taxonomic studies of date plum (*Diospyros lotus* L.) using morphological traits and SCoT markers [J]. *Biochem Syst Ecol*, 61:390-398.

YANG J, WANG P, SHI L, 2016. Optimization of SCoT-PCR reaction system and primer screening for seed watermelon [J]. *NW Agric J*, (6):889-896. [杨静, 王萍, 石磊, 2016. 籽用西瓜 SCoT-PCR 反应体系优化及引物筛选 [J]. 西北农业学报, (6):889-896.]

ZHOU YQ, YAO HL, ZHOU CE, et al., 2010. Research progress of *Rehmannia* breeding [J]. *Guihaia*, 30(3):373-376. [周延清, 姚换灵, 周春娥, 等, 2010. 地黄育种研究进展 [J]. 广西植物, 30(3):373-376.]

ZHOU YQ, WANG WS, WANG XN, et al., 2015. Progress in DNA molecular markers and gene functions of *Rehmannia glutinosa* [J]. *Chin J Plant Sci*, 50(5):665-672. [周延清, 王婉珅, 王向楠, 等, 2015. 地黄 DNA 分子标记与基因功能研究进展 [J]. 植物学报, 50(5):665-672.]

Note: Figure translations are in progress. See original paper for figures.