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Original Article

MYB polymorphism molecular marker: A novel molecular marker for authenticity and geographical origin identification of *Citri Reticulatae Pericarpium*

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ABSTRACT

Objective: *Citri Reticulatae Pericarpium* (Chenpi, CRP) is one of the most used traditional Chinese medicines with great medicinal, dietary and collection values, among which the *Citrus reticulata* cv. 'Chachi' (*Citrus reticulata* cv. Chachiensis) from Guangdong Xinhui is the geoherb of CRP. Xinhui CRP in the market was often counterfeited with other varieties or origins, molecular identification method can effectively distinguish different CRP varieties, but it's still a difficult problem to identify the same CRP variety from different origin. It is necessary to discover a new molecular marker to ensure the safe and effective application of Xinhui CRP.
Methods: We selected one of the most studied transcription factor families in *Citrus* genus, MYB, to design the specific candidate primers on the conserved region. The primers with good band repeatability and high polymorphism were screened for PCR amplification of the test materials, and the genetic similarity coefficient among different families, genera, species, and origins were calculated. The cluster analysis was performed by unweighted pair group method using arithmetic average (UPGMA).
Results: A total of ten MYB primers were screened out to identify Xinhui CRP from plants from different family (*Panax ginseng* and *Morus alba*), genus (*Clausena lansium* and *Zanthoxylum schinifolium*), and species (*Citrus reticulata*, *C. sinensis* and *C. maxima*). Furthermore, two from the ten primers, M1 and M10, were found to distinguish Xinhui CRP from other origins. There were 169, 113, 133 and 134 polymorphic bands in the identification of different families, genera, species, and origins respectively, and the accordingly polymorphism ration were 79.88%, 76.87%, 79.20% and 82.84%. Moreover, M1 was discovered to be the best primer to identify Xinhui CRP from other seven origins, the cluster analysis results based on the genetic similarity coefficients were consistent with the geographical distribution.
Conclusion: This study established a novel molecular identification method according to MYB transcription factor, which can analyze the potential parental relationship of CRP germplasm, as well as identify the quality and origins of Xinhui CPR.

1. Introduction

Citri Reticulatae Pericarpium (Chenpi, CRP) is a widely used traditional Chinese medicine (TCM) with a long history, which had a variety of effects including antitussive, antioxidant, anti-cardiovascular and anti-tumor (Yu et al., 2018; Song et al., 2022; Zou et al., 2022). The main sources of CRP are the dried and mature peels of *Citrus reticulata* Blanco and its cultivated varieties, such as *C. reticulata* cv. 'Chachi', *C. reticulata* cv. 'Dahongpao', *C. reticulata* cv. 'Unshiu', *C. reticulata* cv. 'Tangerina' (Li et al., 2019). The main producing areas of CRP are Guangdong, Fujian, Sichuan, Guangxi and other places in China, while the producing area unanimously

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recognized by the market is Xinhui subdistrict of Guangdong province. Xinhui CRP uses the variety of *C. reticulata* cv. 'Chachi', which is regarded as the geoherb. Geoherb refers to medicinal materials with suitable origin, high quality, sophisticated processing, outstanding curative effect, and regional characteristics formed by certain medicinal biological species under the comprehensive action of specific natural conditions and ecological environment. Xinhui CRP has edible, medicinal and even a certain collection value. The industrial output value of Xinhui CRP reached 8.5 billion yuan in 2019, and the brand value exceeded 10 billion yuan. In recent years, the rapid development of Chinese medicine cultivation industry made many CRP from different cultivars circulate in the market. Due to the limitation of the Xinhui production area, the output of Xinhui CRP does not meet the demand for food and medicines, resulting in counterfeiting of Xinhui CRP in the market. Since Xinhui CRP has no characteristic compounds (Zheng et al., 2020), it is difficult to identify Xinhui CRP for drugs that cannot be visually detected, such as injections and Chinese patent medicines, and molecular identification has certain advantages in this respect.

It's difficult to make the rapid classification and identification of TCMs using traditional methods of chemical composition and appearance, and the identification process itself is prone to error. With the rapid development of bioinformatics, molecular identification methods have been developed because of their advantages in the speed and accuracy of TCM identification (Han, Wang, Zhang, & Wang, 2018). DNA molecular markers are widely used in species identification, animal and plant genetic breeding, genome mapping, gene bank construction, gene cloning and many other aspects due to their remarkable advantages (Mishra et al., 2016; Nadeem et al., 2018). In recent years, many innovative molecular markers focused on targeted DNA sequences have been developed, including differential display reverse transcription polymerase chain reaction (DDRT-PCR) (Huang et al., 2015), start codon targeted polymorphism (SCoT) (Rai, 2023), inter retrotransposon amplified polymorphism (IRAP) (Ghaffarian & Mohammadi, 2023), retrotransposon microsatellite amplified polymorphism (REMAP) (Roy, Choi, Lee, & Kim, 2015). Retrotransposon based fingerprinting methods, IRAP and REMAP, had successfully developed DNA molecular markers for rapid characterization of *Citrus* and its related genera *Fortunella* and *Ponciru*, and IRAP based on the first complete retrotransposon identified in *Citrus* (*CIRE1*) could further identify closely related accessions of *Citrus sinensis* (Biswas, Baig, Cheng, & Deng, 2010; Horibata and Kato, 2020). Furthermore, the SCoT molecular markers have been proven to be useful in the characterization identification and structuring of genetic diversity of the species in *Citrus* (Mahjbi, Baraket, Oueslati, & Salhi-Hannachi, 2015). Therefore, DNA molecular markers have a great potential in precise identification of the genus *Citrus* and related genera. CRP is a medicinal plant derived from *Citrus,* the above molecular methods can be used to distinguish CRP from other *Citrus* species, but there is still no molecular marker to identify CRP from different geographical origins.

MYB transcription factor family is called one of the largest and most widely distributed transcription factor families among plants, which is involved in the gene regulation of various developmental and morphological processes, metabolic pathways and signaling pathways (Thakur and Vasudev, 2022). And MYB is also the multi-domain protein, which is comprised of three domains including conserved N-terminal domain, central domain, and variable C-terminal domain (Biedenkapp, Borgmeyer, Sippel, & Klempnauer, 1988). Each N-terminal domain generally consists of 1 to 4 incomplete repeats (R structure), and each R structure is composed of about 50–53 highly conserved amino acid residues that encode three helices, forming a helix-turn-helix structure, through which MYB protein can be inserted into the target DNA to regulate the expression of the target gene (Dubos et al., 2010). The MYB domain was designed primers due to its conserved, differences between different varieties and origins of CRP are shown through low conservation. In the long process of natural selection and evolution, CRP from different varieties and different producing areas formed its unique gene expression and regulation mechanism, which was shown in low conservation. So that *MYB* gene family polymorphism markers have great potential in the identification of CRP. There were 100 *MYB* genes identified from the *Citrus sinensis* genome (Liu, Wang, Xu, Deng, & Xu, 2014), and the functions of several *MYB* genes have been found to be involved in the formation of flavor and color (Huang et al., 2020; Zhang et al., 2020; He et al., 2022). Although there are relatively few studies on MYB in *C. reticulata*, previous study has found that CrMYB68 was an important regulator of carotenoid metabolism and abscisic acid (ABA) biosynthesis (Zhu et al., 2017). Therefore, *MYB* is a very important family of transcription factors with multiple gene members in *Citrus,* which is a potential gene for development into molecular markers. In addition, in the identification of the geographical origin of *Citrus* plants and other TCMs, chemical identification methods still occupy a mainstream position, such as the application of GC-MS and near infrared spectroscopy technology (Yin et al., 2019; Long et al., 2022; Zhang et al., 2024), the molecular identification methods for the geographical origins of CRP and TCM still need to be developed. Based on previous molecular marker studies, the feasibility of identification of CRP origin based on *MYB* gene molecular marker will also be further explored.

According to the characteristics of multi-domain and conserved region sequences in CRP *MYB* genes, this study raises a hypothesis to develop a new DNA molecular marker based on *MYB* transcription factor genes, which can not only identify CRP from different families, genera and species, but also distinguish CRP from different geographical origins (Fig. 1). The development of this method is based on the differentiation results of DNA sequence polymorphism of *MYB* gene family in plants of different families, genera, species and origins, aims to explore its potential as a novel molecular marker of CRP polymorphism, to further verify the feasibility of *MYB* gene family polymorphism marker in the identification of CRP geographical origin, so as to expand the application range of molecular markers in TCM origin identification. Furthermore, this new molecular identification method can produce plenty of potential DNA polymorphic markers, which can be used for genetic diversity analysis, germplasm identification, and phylogenetic analysis. And CRP would be regarded as an important TCM representative to validate this method and provide a basis for the authenticity identification, source identification and resource protection of the herbal medicines in the future.

2. Materials and methods

2.1. Plant material

The plant material consists of samples from different families, genera, species, and CRPs of seven geographical origins. The tested samples of different families included *Panax ginseng* C. A. Mey. (PG, family: Araliaceae) and *Morus alba* L. (MA, family: Moraceae), and the tested samples of different genera comprised *Clausena lansium* (Lour.) Skeels (CL, family: Rutaceae) and *Zanthoxylum schinifolium* Sieb. et Zucc. (ZS, family: Rutaceae). In the *Citrus* genus, three common species including *Citrus reticulata* Blanco, *Citrus sinensis* (L.) Osbeck and *Citrus maxima* (Burm.) Merr. were selected for test. For the identification of different CRP geographical origins, besides the sample from the genuine producing area of Xinhui District of Guangdong province (XH), other samples were from Japan (JP)

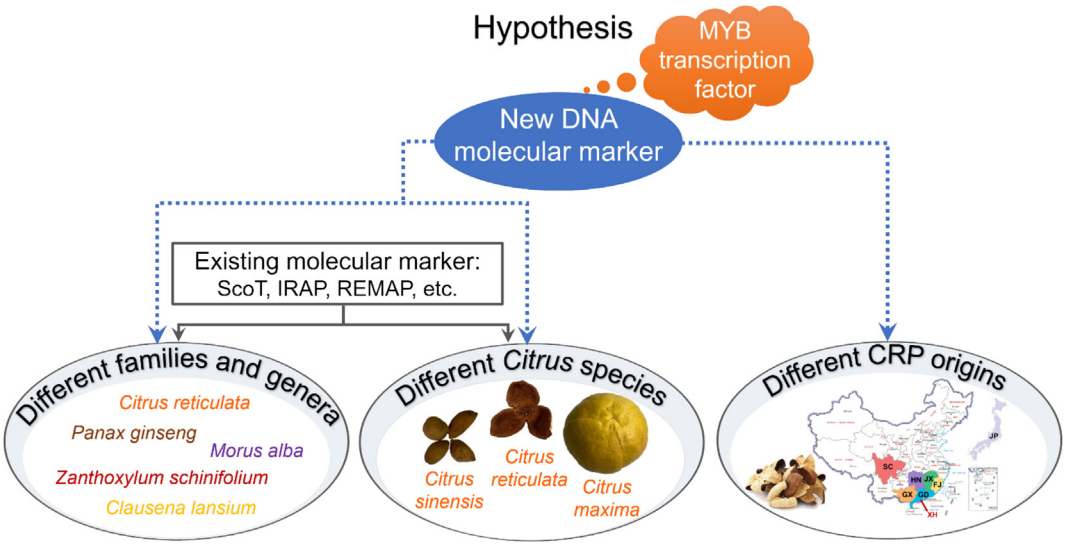

Fig. 1. Research hypothesis of developing MYB transcription factors as new DNA molecular markers. The map examination and approval number is GS(2019)1676.

and six provinces in China including Guangdong (GD), Guangxi (GX), Jiangxi (JX), Sichuan (SC), Hunan (HN) and Fujian (FJ) provinces. The GD CRP sample from Guangdong province was another location other than Xinhui. All the samples were identified by Dr. Chunsong Cheng, an experienced researcher of Chinese medicines in Lushan Botanical Garden of Chinese Academy of Science. The detailed information of collected samples in this study were shown in the following Table 1.

2.2. DNA extraction

DNA was extracted using modified cetyl trimethyl ammonium bromide (CTAB) method (Porebski et al., 1997; Bustamante et al., 2019). CRP was powdered by the German QIAGEN Tissuelyser II high-throughput tissue grinder (Qiagen, Hilden, Germany) with liquid nitrogen, followed by incubating at 65 °C for 2 h in HH-4 digital display constant temperature water bath with intermittent shaking after mixing with CTAB extraction buffer and β-mercaptoethanol. The above mixture was treated with phenol: chloroform: isoamyl alcohol mixture (25:24:1) extraction. The supernatant was added one volume of ice-cold isopropanol to precipitate for 1 h, washed with solution containing 75% alcohol twice. Then the precipitate was air-dried and added water to dissolve it. Finally, a total of 200 μL sterile water was added to dissolve the precipitate. The concentration and purity of isolated DNA was determined by Thermo Scientific NanoDrop spectrophotometer 2000c UV-Visible Spectrophotometer (Thermo Scientific, Waltham, USA). The quality of all the DNA samples were evaluated by measuring their concentration and optical density ratio (Table S1), and the DNA samples that met the standard were applied for the following experiments.

2.3. Gene structure and sequence features of MYBs in Citrus

For building the 3D model for *Citrus* MYB protein, the target amino acid sequence information was submitted to the online SWISS-MODEL server for establishing (http://swissmodel.expasy.org/), template with the highest quality and similarity was selected for model building (Arnold et al., 2006; Kiefer et al., 2009). The predicted 3D model was downloaded and used for further analysis. To demonstrate conservation of MYB domain at particular positions, WebLogo (https://weblogo.berkeley.edu/) was used to investigate further, especially tryptophan residues (W) in R structures. The conserved motifs of MYBs in *Citrus* were identified by MEME (suite version 5.5.5, http://meme-suite.org/), and the parameters for performing this analysis were as follows: number of repetitions = any; maximum number of motifs = 8 (for protein) and 18 (for DNA); optimum motif length = 6–50 amino acids.

Table 1
Samples for CRP molecular identification method establishment based on *MYB* genes in this study.

Identification classification	Code	Species names	Origins
Genuine producing area	XH	*C. sinensis* cv. 'Chachi'	Huangchong Village, Yamen Town, Xinhui district, Jiangmen City, Guangdong Province, China
Different families	PG	*P. ginseng*	Xinkai River, Ji'an City, Jining Province, China
	MA	*M. alba*	Taipa, Macao SAR, China
Different genera	CL	*C. lansium*	Purchased in Herbal medicine market of Macao SAR, China
	ZS	*Z. schinifolium*	Purchased in Taobao
Different species	CS	*C. sinensis*	Purchased in Herbal medicine market of Macao SAR, China
	CM	*C. maxima*	Purchased in Herbal medicine market of Macao SAR, China
Different geographical origins	GD	*C. reticulata* cv. 'Chachi'	Shuikou Town, Kaiping City, Guangdong Province, China
	GX	*C. reticulata* cv. 'Chachi'	Pubei County, Qinzhou City, Guangxi Province, China
	JX	*C. reticulata* cv. 'Ponkan'	Nanchang City, Jiangxi Province, China
	SC	*C. reticulata* cv. 'Dahongpao'	Anyue County, Ziyang City, Sichuan Province, China
	HN	*C. reticulata* cv. 'Succosa'	Shimen County, Changde City, Hunan Province, China
	FJ	*C. reticulata* cv. 'Tangerina'	Ningde City, Fujian Province, China
	JP	*C. reticulata*	Akitsu, Hiroshima, Japan

2.4. Primer design

In principle, this molecular marker is similar to SCoT (Collard and Mackill, 2009), ISSR (Blair, Panaud, & McCouch, 1999) or single primer amplification reaction. The sequences of the designed primers were from the National Center for Biotechnology Information (NCBI) website, by searching the *MYB* gene family of *C. reticulata* Blanco. The searched sequences were put into the CD-search function on the NCBI webpage to find the conserved gene region in the sequence. To prevent errors or sample sizes that are too small, sequences of positive and negative 50 bp functional regions were blasted with *MYB* genes of *Rutaceae* plants to find homologous regions. Finally, homogeneous regions were used to design primers by Primer Premier 5.0. The sequences of primers were synthesized by BGI Write Gene Technology Co., Ltd..

2.5. Identification of plants from different families, genera, and species

The molecular identification based on MYB DNA markers were conducted by the following steps. Firstly, PCR reactions were carried out with genome DNA templates of three different families: Rutaceae (XH), Araliaceae (PG) and Moraceae (MA) on MYB primers. Then, three different genera of Rutaceae: *Citrus* (XH), *Clausena* (CL), and *Zanthoxylum* (ZS) were applied by PCR reactions with MYB primers. Thirdly, different species of *Citrus*: *C. reticulata* (XH), *C. sinensis* (CS), and *C. maxima* (CM) were applied by PCR reactions with MYB primers. PCR reaction volume was 25.0 μL, containing 1.0 μL of template DNA (10 ng/μL), 1.0 μL primer at 1 OD, 12.5 μL 2× Power Taq PCR Master Mix, and 10.5 μL ddH_2O. PCR was performed on Applied biosystems Veriti 96 well Thermal cycler PCR (Applied Biosystems, Foster City, CA, USA). Initial denaturation was carried out at 98 °C for 2 min, followed by 40 cycles of 98 °C for 10 s, annealing for 15 s, extension at 72 °C for 2 min, and final extension at 72 °C for 10 min. Annealing temperature depended on each primer. After amplification, 1.0 μL of 6× loading buffer was added to 6.0 μL PCR products. The reaction mixture was separated in 1.0% agarose gels containing 10 000× AidRed (GelRed) through electrophoresis in 1× TAE buffer solution at 110 V and visualized under Bio-Rad Gel Doc XR + ultraviolet transilluminator.

2.6. Identification of CRP from different geographical origins

To explore whether the MYB primers could identify CRP from seven different geographical origins, MYB primers were screened. Seven different origins of CRP (Fig. 2) were from Xinhui district of Guangdong province, Chaozhou city of Guangdong province, Guangxi province, Jiangxi province, Sichuan province, Hunan province, Fujian province and Japan, and their corresponding codes were XH, GD, GX, JX, SC, HN, FJ and JP. The PCR identification and DNA polymorphism bands electrophoresis methods were referred to 2.4.

2.7. Data analysis

The electrophoresis results of PCR amplified products were assembled and recorded the position of clear and easily identifiable

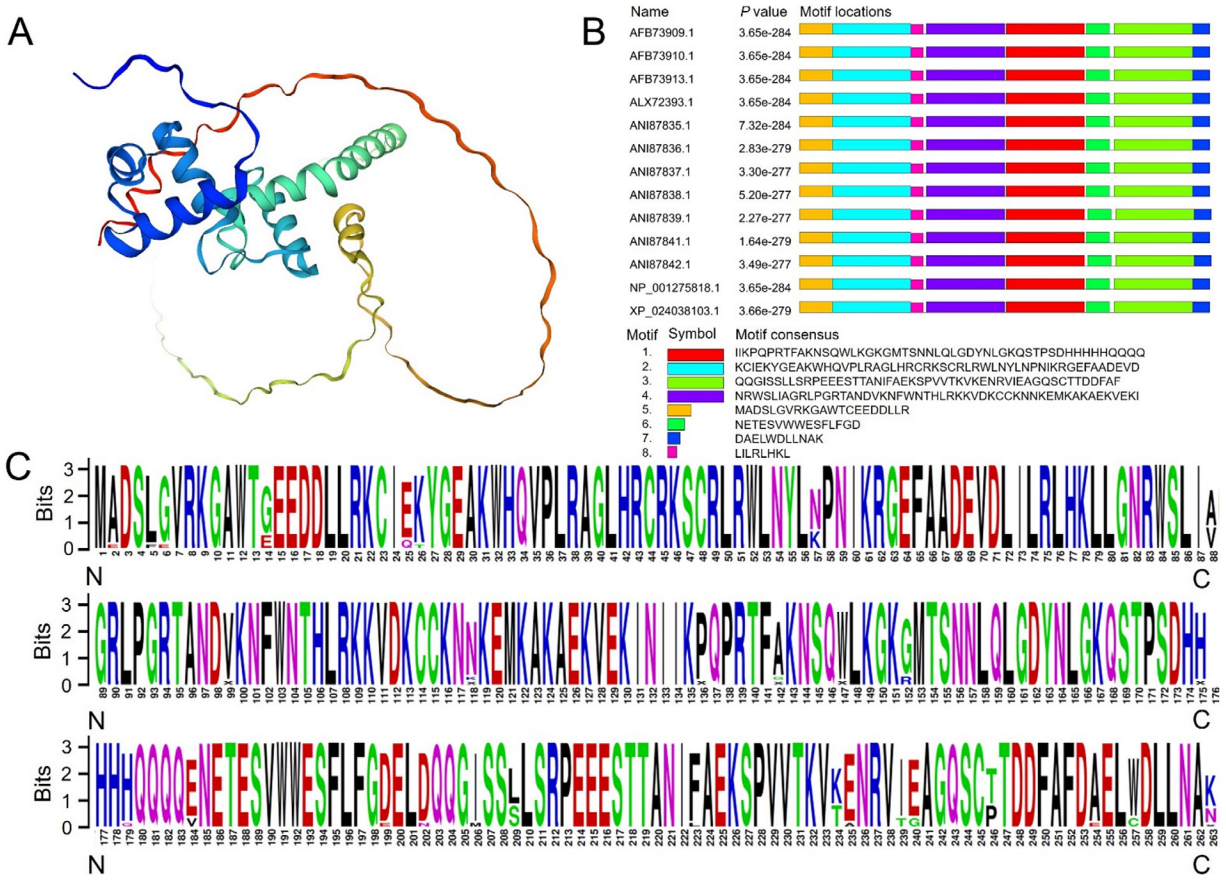

Fig. 2. Characterization of MYB protein sequences in *Citrus* plants. (A) Tertiary structure prediction of *Citrus* MYB protein. The most suitable template for it was from SMTL ID: A0A191TGK7.1.A. (B) Conserved motif analysis of MYB proteins in different *Citrus* plants. AFB73909.1, AFB73910.1, AFB73913.1, AFB72393.1, NP_001275818.1 and XP_024038103.1 were MYB proteins from *C. sinensis*, ANI87841.1 and ANI87842.1 were MYB proteins from *C. reticulata*, ANI87835.1, ANI87836.1, ANI87837.1, ANI87838.1 and ANI87839.1 were MYB proteins from *C. maxima*, *C. medica* L., *C. indica* Tanaka, *C. cavaleriei* H. Lév. ex Cavalier and *C. micrantha* Wester, respectively. (C) The MYB domain consensus of *Citrus* plants by WebLogo.

bands using the '0/1' system. Presence of a band was recorded as '1' and absence of a band was recorded as '0'. In order to ensure the quality, selecting the bands within the range of 100–3 000 bp in the area with many bands for statistics, and establishing the MYB marker matrix. Polymorphic index content (PIC) was calculated as follows: PIC= $1-\sum P_i^2$ where P_i is the band frequency of the i^{th} allele. The Resolving Power (Rp) was used to evaluate the ability of the primers to distinguish between genotypes, and the formula Rp = $\sum I_b$ where band informativeness $I_b = 1 - (2 \times |0.5 - p|)$ and p is the proportion of genotypes containing band I. The comparisons of plants in different families, genera and species, based on the proportion of polymorphism bands produced by the primers, were calculated using the Dice similarity coefficients in the NTSYS pc 2.10e program (Collard and Mackill, 2009). Cluster analysis was using unweighted pair group method with arithmetic averages (UPGMA) to show a phenetic representation of genetic relationships as revealed by the similarity coefficient. Wekemo web version software was performed to Orthogonal partial least squares discriminant analysis (OPLS-DA) mapping analysis based on 1/0 data matrix.

3. Results

3.1. Protein characteristic analysis of Citrus MYB transcription factors

MYB was one of the largest and most important transcription factor families in plant, and it also played the significant role in the response to abiotic and biotic stresses. There were five *MYB* genes of *C. reticulata* Blanco found on the NCBI database. In order to find the conserved region of the sequence, CD-search is performed on the sequence. The six conserved regions found were PLN03212 (transcription suppressor MYB5), REB1 (MYB superfamily proteins, including transcription factors and mRNA splicing factors) and four segments of MYB-DNA-binding regions. As the number of *C. reticulata MYB* gene was limited, *C. reticulata MYB* transcription factor (Ruby) gene (GenBank Accession No. KT591674.1) was the most complete gene with 3 540 bp length, so it was selected from these five genes as the template to conduct the basic local alignment search tool (BLAST) alignment, in order to find the MYB homologous region of *Citrus* plants. By inputting the conserved *Citrus* MYB protein domain sequences in the online soft-

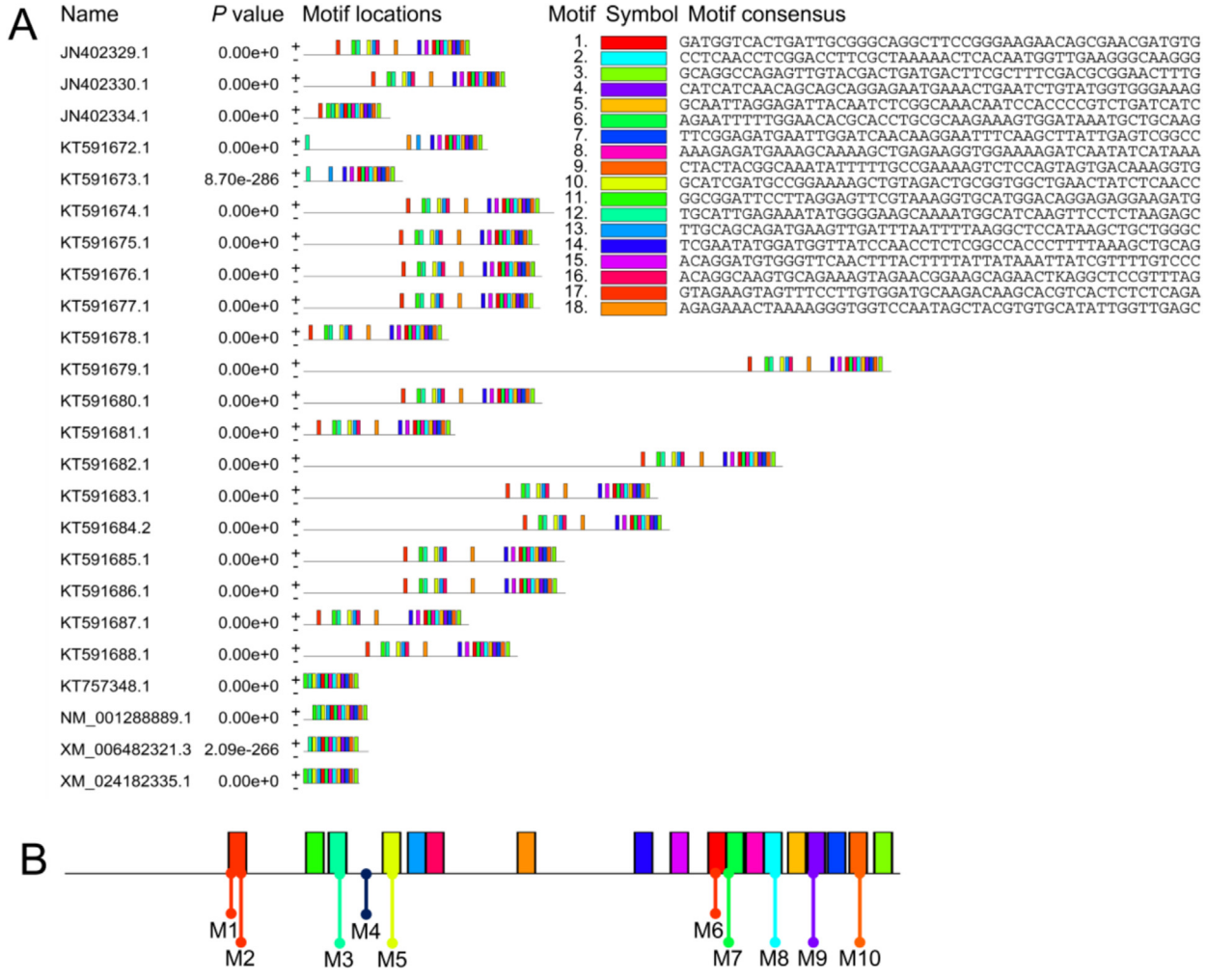

Fig. 3. MYB polymorphic primers designed based on conserved *Citrus* MYB DNA sequences. (A) Conserved motif analysis of MYB DNA sequences in different *Citrus* plants. JN402329.1, JN402330.1, JN402334.1, KT757348.1, NM_001288889.1 and XM_006482321.3 were MYB DNAs from *C. sinensis*, KT591675.1, KT591676.1, KT591677.1 and KT591678.1 were MYB DNAs from *C. medica*, KT591682.1 and KT591683.1 were both MYB DNAs from *C. australasica*, KT591685.1 and KT591686.1 were both MYB DNAs from *C. trifoliata*, KT591672.1, KT591673.1, KT591674.1, KT591679.1, KT591680.1, KT591681.1, KT591684.2, KT591687.1, KT591688.1 and XM_024182335.1 were MYB DNAs from *C. reticulata* cv. 'Unshiu', *C. sunki* Hort. ex Tanaka, *C. reticulata*, *C. medica*, *C. indica*, *C. cavaleriei*, *C. micrantha*, *C. japonica* var. *margarita* (Loureiro) Guillaumin, *C. swinglei* Burkill ex Harms and *C. clementina* Hort. ex Tanaka, respectively. (B) The detailed location of selected 10 MYB polymorphic primers according to conserved DNA motifs.

ware SWISS-MODEL (https://swissmodel.expasy.org/), the analysis result showed it was most similar with the structure of *Citrus trifoliata* (L.) Raf. MYB transcription factor AlphaFold DB model (SMTL ID: A0A191TGK7.1.A.) than other templates. Protein secondary structure prediction result showed that the 3D protein structure of a CRP MYB was composed of α-helix, β-corner, β-turn and random coil, which were the main secondary components of MYB transcription factors. Among them, α-helix and random coil had a larger proportion than β-corner and extended chain structure (Fig. 2A). The structure of α-helix was relatively stable, and the random coil was mostly located on the protein surface, which was easy to form DNA binding domain. This proportion was consistent with the helix-helix-turn-helix structure of the MYB domain, which was easy to bind DNA specifically and then regulate DNA transcription, thereby enhancing or inhibiting gene expression. And these conserved regions were tested among the *Citrus* plants, such as *C. sinensis*, *C. maxima*, *C. medica*, *C. trifoliata*, the motif analysis results by MEME (Fig. 2B) and the detailed MYB amino acid domain consensus result by WebLogo (Fig. 2C) showed these MYB amino acid domains were highly conserved. Therefore, the *MYB* genes belonged to *Citrus* plants had the certain conserved gene motifs. Based on these motifs, the specific primers could be designed to amplify the polymorphic sequences, so as to establish a new molecular identification method and discover corresponding markers for identifying the authenticity and source of CRP.

Table 2
Designed primers of MYB molecular markers of *C. reticulata* Blanco.

Primer No.	Sequence (5' to 3')
M1	CAAGACAAGCACGTCACT
M2	TCCTTGTGGATGCAAGAC
M3	GCAAAATGGCATCAAG
M4	TCACAGGAGCTGCTTCTA
M5	GCTGTAGACTGCGGTGGC
M6	TGATTGCGGGCAGGCTTC
M7	ACAGCGAACGATGTGAAG
M8	TCAACCTCGGACCTTCGC
M9	CAACAGCAGCAGGAGAAT
M10	GCCGAAAAGTCTCCAGTA

3.2. DNA motif analysis of Citrus MYBs and polymorphic primers screening

As the protein sequences of *Citrus* MYBs were highly conserved, the corresponding DNA sequences were also conducted the MEME motif analysis, the results showed there were 18 conserved DNA motifs, which were existed among a variety of *Citrus* plants, including *C. reticulata*, *C. sinensis*, *C. maxima*, *C. medica*, *C. trifoliata*, *C. australasica* and so on (Fig. 3A). Through the analysis of sequences from the *Citrus MYB* genes, especially the DNA sequence from *C. reticulata* Blanco (GenBank Accession No. KT591674.1), to obtain the corresponding *MYB* gene sequences of samples, MYB polymorphic primers were designed based on the homologous region. A total of designed 21 polymorphic molecular marker primers were tested to screen the most suitable primers (Fig. S1). Among those 21 primers, the DNA electrophoresis results showed that 10 primers with clear bands and obvious spacing were selected and named M1 to M10, and most of these ten primers were located at the conserved DNA motifs to ensure the stability of this new molecular identification method (Fig. 3B). Then, these 10 primers were applied to the following identification of different families, genera, species and CRP geographical origins. The detailed primer sequences were showed in Table 2.

Table 3
Statistical results of MYB primers on identification of different families, genera and species.

Identification	Primer names	Total bands	No. of polymorphic bands	Percentage of polymorphism (%)	PIC	Rp
Different families	M1	19	15	78.95	0.932 2	10.000 0
	M2	17	13	76.47	0.921 8	8.000 0
	M3	10	7	70.00	0.872 0	4.666 7
	M4	19	16	84.21	0.937 5	10.666 7
	M5	18	15	83.33	0.933 6	9.3333
	M6	21	20	95.24	0.944 1	13.333 3
	M7	17	15	88.24	0.934 4	10.000 0
	M8	16	13	81.25	0.924 6	8.666 7
	M9	15	7	46.67	0.921 9	4.666 7
	M10	17	14	82.35	0.926 0	9.333 3
	Average	16.9	13.5	79.88	–	–
	Total	169	135	–	–	–
Different genera	M1	16	14	87.50	0.920 1	8.000 0
	M2	19	16	84.21	0.932 2	10.000 0
	M3	11	9	81.82	0.882 8	6.000 0
	M4	16	14	87.50	0.922 5	9.333 3
	M5	15	12	80.00	0.917 2	7.333 3
	M6	13	13	100.00	0.914 1	8.666 7
	M7	15	11	73.33	0.919 1	7.333 3
	M8	14	9	64.29	0.913 6	6.000 0
	M9	13	5	38.46	0.916 0	3.333 3
	M10	15	10	66.67	0.914 2	6.666 7
	Average	14.7	11.3	76.87	–	–
	Total	147	113	–	–	–
Different species	M1	12	8	66.67	0.896 7	5.333 3
	M2	19	17	89.47	0.937 0	11.333 3
	M3	10	7	70.00	0.872 0	4.666 7
	M4	17	14	82.35	0.926 0	9.333 3
	M5	17	16	94.12	0.932 0	10.666 7
	M6	6	5	83.33	0.790 1	3.333 3
	M7	13	9	69.23	0.837 4	5.333 3
	M8	13	12	92.31	0.911 6	8.000 0
	M9	12	7	58.33	0.908 2	4.000 0
	M10	14	9	64.29	0.913 6	6.000 0
	Average	13.3	10.4	78.20	–	–
	Total	133	104	–	–	–

3.3. Identification of plants from different families, genera and species

A representative set of plants from different families, genera, and species were used to initially evaluate the *MYB* gene family markers that can be used for plant identification. There were obvious differences between different families and different genera. In the identification of different species, only primers M6 and M7 had the same result of the bands, other primers had obvious differentiated polymorphic bands.

The identification results and agarose gel electrophoresis bands of different families, genera and species were shown in Table 3 and Fig. 4. In identification of different families, totally 169 bands were obtained, among which 135 were polymorphic bands. The percentage of polymorphic sites accounted for 79.88%. The amplification products using 10 primers ranged from 46.67% to 95.24% in producing polymorphic bands. Rp values of the *MYB* gene family markers ranged from 4.666 7 to 13.333 3 and PIC values were found to be 0.872 0–0.944 1. These two parameters provided a baseline that helped determine the validity of the primers used in the fingerprint identification process, thereby determining the originality of the technique (Bhattacharyya, Kumaria, Kumar, & Tandon, 2013). For different genera, a total of 147 bands were obtained, among which 113 were polymorphic bands. The percentage of polymorphic sites accounted for 76.87%. The amplification products using 10 primers ranged from 38.46% to 100.00% in producing polymorphic bands. Rp values ranged from 3.333 3 to 10.000 0. PIC values varied from 0.882 8 to 0.932 2, indicated that these loci were highly informative. In identification of three different species belonged to *Citrus*, a total of 133 bands were obtained, among which 104

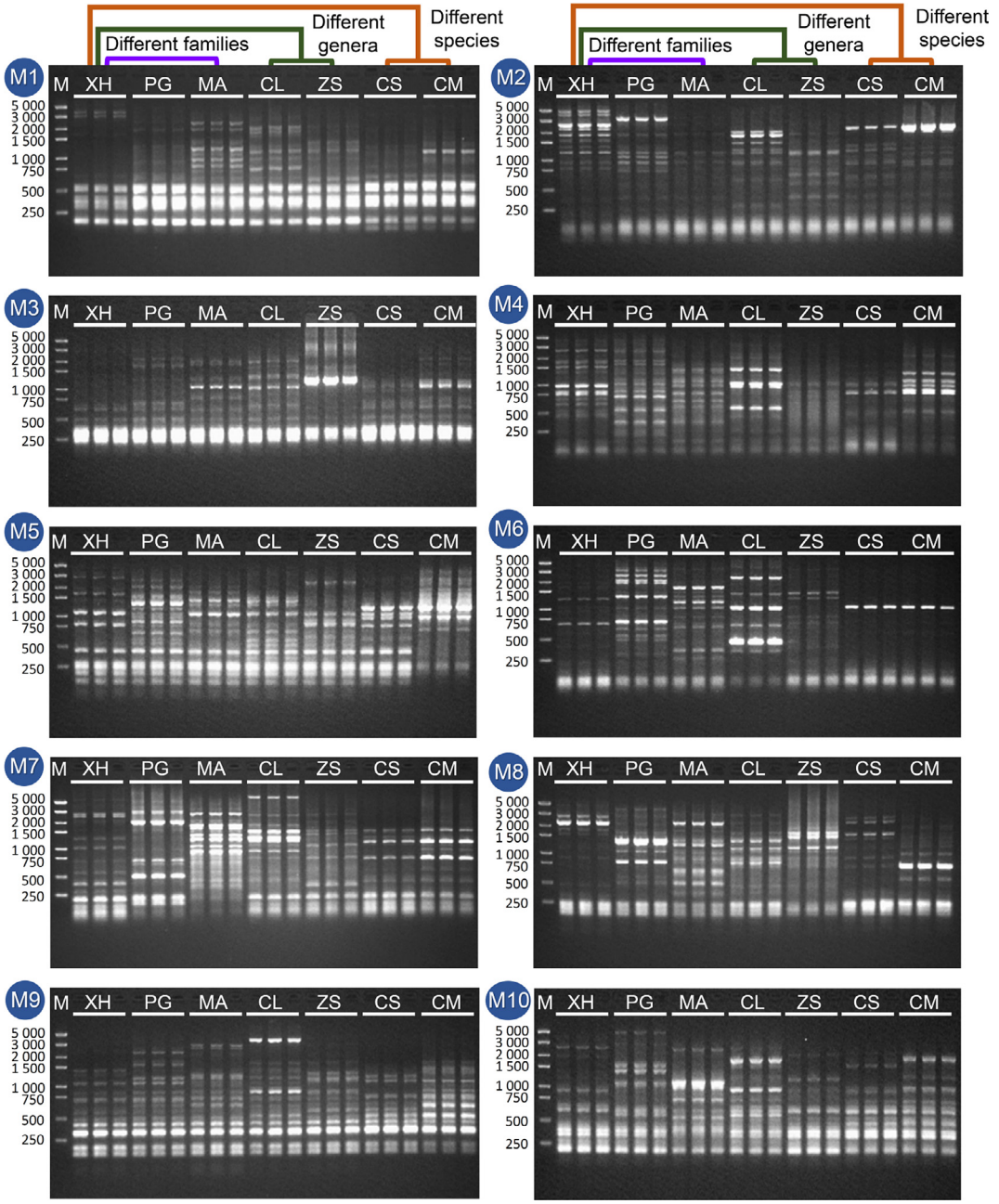

Fig. 4. Electrophoresis results of MYB polymorphic primers to identify different families, genera, and species. XH, PG, MA, CL, ZS, CS and CM represented samples of Xinhui *C. reticulata*, *P. ginseng*, *M. alba*, *C. lansium*, *Z. schinifolium*, *C. sinensis* and *C. maxima* respectively. M represented maker, and the number on the left side of electrophoresis gel was the band size, of which the unit was bp.

were polymorphic bands. The percentage of polymorphic sites accounted for 79.20%. The amplification products using 10 primers ranged from 58.33% to 94.12% in producing polymorphic bands. Rp values ranged from 3.333 3 to 11.333 3. The maximum and lowest PIC values were 0.937 0 for M2 and 0.790 1 for M6 respectively.

The UPGMA cluster analysis and the similarity coefficient matrix of all the 10 MYB primers were shown in Fig. 5. From the results, basically all the 10 primers could be applied to separate plants of different families, genera and species successfully. Except that M6 primer could not distinguish *C. sinensis* and *C. medica*, and M9 could not distinguish *C. medica* and *Z. schinifolium*. The similarity coefficients between XH CRP and other species (PG, MA, CL, ZS, CS and CM), which calculated as the average of corresponding coefficients in all the 10 primers (Fig. 5A–J), were 0.478 9, 0.508 2, 0.497 4, 0.576 3, 0.536 5 and 0.432 1 respectively. As all of them were relatively low, these primers should have a high success rate in identifying XH CRP from other species.

3.4. Identification of CRP from different origins

As the MYB molecular markers have been proven to be successful on the identification of different families, genera and species, the further identification exploration of different CRP geographical origins was conducted. The relevant analysis results showed that successful MYB polymorphism markers for different origins of CRP were discovered to be M1 and M10, which could distinguish eight different origins from each other (Fig. 6). And other primers M3, M4, M5, M6, M7, M8, M9 could distinguish Xinhui CRP from other origins, primer M2 had the lowest identification success rate that couldn't identify these CRPs from different origins (Figs. S2–S5). And the primers were also applied to different batches of Xinhui CRP, and the verification results showed that the stability was qualified (Fig. S6).

The identification data of different geographical origins were shown in Table 4. A total of 134 bands were obtained, among which 111 were polymorphic bands. The percentage of polymor-

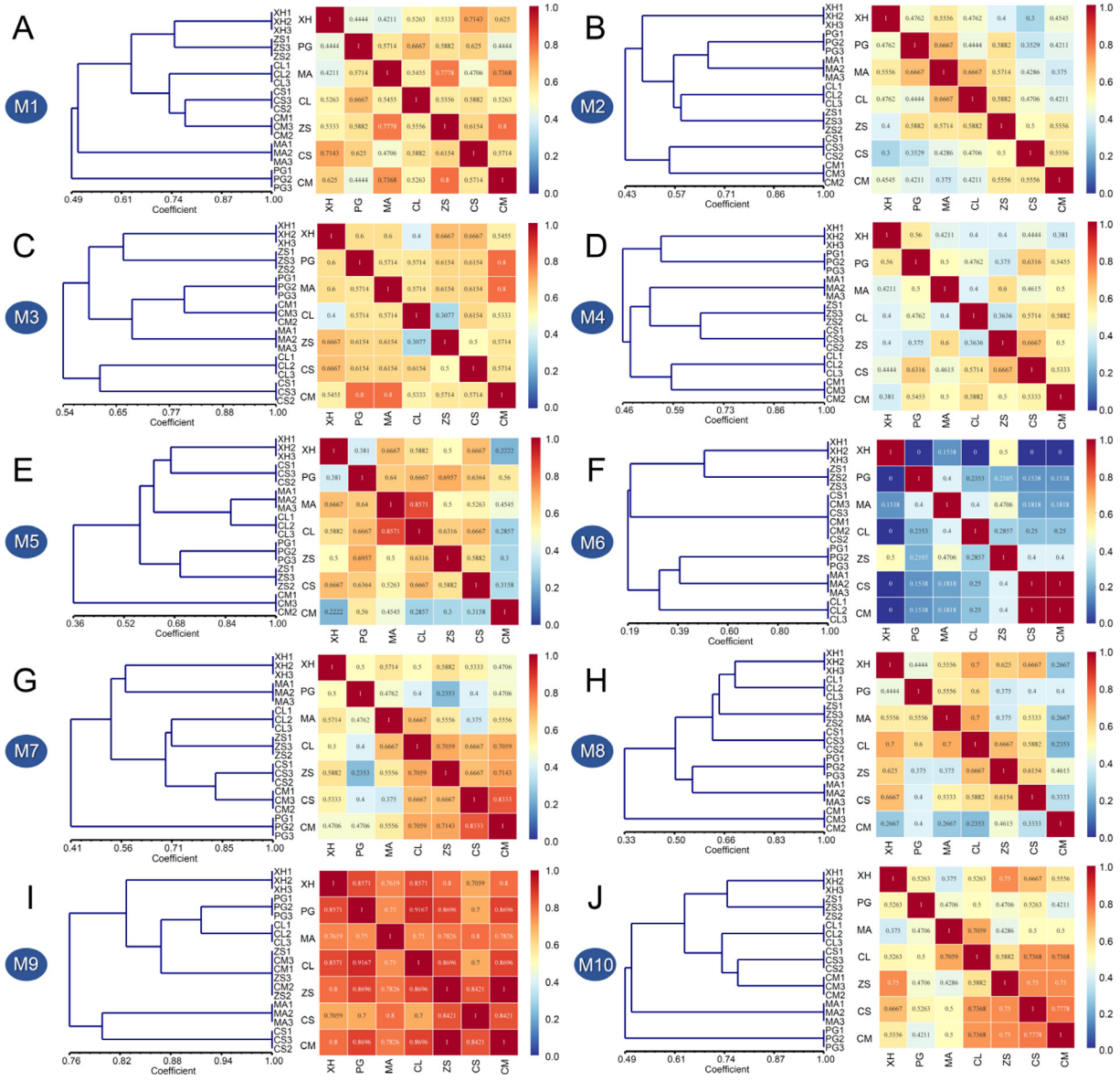

Fig. 5. UPGMA cluster analysis and similarity coefficient matrixes of MYB polymorphic primers on identification of different families, genera and species. A–J represented UPGMA cluster results and similarity coefficient matrixes of M1–M10 respectively.

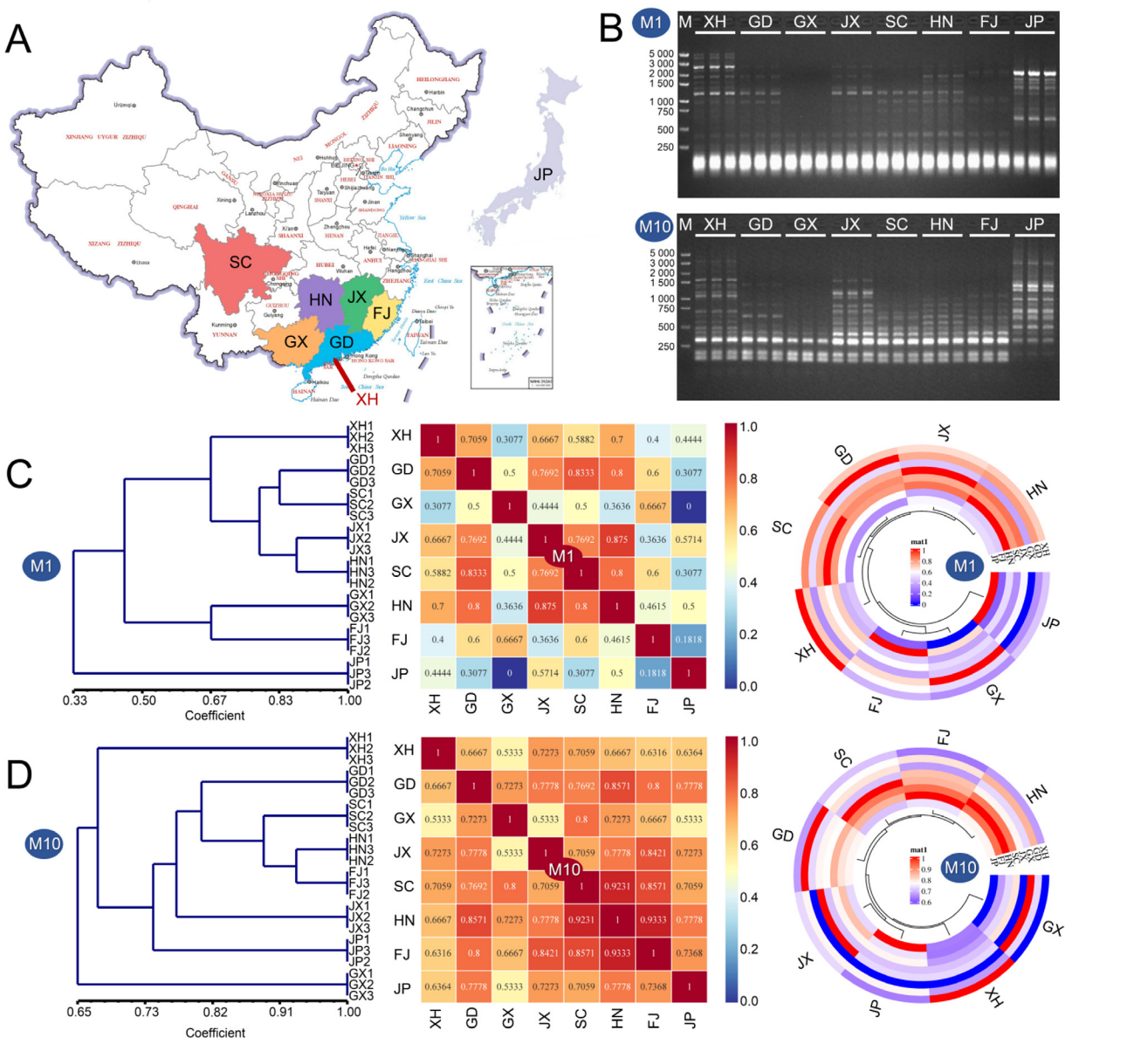

Fig. 6. Identification results of MYB polymorphic primers to distinguish different CRP geographical origins. (A) The detailed geographical distribution of CRP [examination and approval number of map is GS(2019)1676]. (B) Electrophoresis results of MYB polymorphic primers M1 and M10 to identify different CRP geographical origins. (C) UPGMA cluster analysis and similarity coefficient matrix of MYB polymorphic primer M1 on identification of different CRP geographical origins. (D) UPGMA cluster analysis and similarity coefficient matrix of MYB polymorphic primer M10 on identification of different CRP geographical origins.

phic sites accounted for 82.84%. The percentage of producing polymorphic bands ranged from 41.67% to 100.00%. Rp values of the *MYB* gene family markers ranged from 2.000 to 7.250 and PIC values were found to be 0.847 7–0.904 5.

As shown in Fig. 6B, C, primer M1 was the best primer to identify different CRP geographical origins, and the similarity coefficients of all the eight origins were between 0.000 0–0.875 0. Moreover, cluster analysis showed that samples from eight geographical origins were divided into eight branches, the origins of XH, GD, SC, JX and HN clustered together, the origins of GX and FJ cluster together, and the origin of JP formed a cluster separately, and the result was basically consistent with the geographical distribution. Primer M10 could also identify the eight CRP origins, but the value of Rp was little lower than M1, and the cluster result showed that the origin of GX formed a branch separately, so the degree of differentiation was relatively weak (in Fig. 6D).

4. Discussion

4.1. MYB is a significant transcription factor family in Citrus plants

MYB molecular marker is based on the single primer amplified region principle. It is similar to SCoT molecular marker, because a single primer is used as the forward and the reverse primer, which is designed according to the homogeneous region, and the design primer regions are on or near the functional gene. Due to the design of primers in the homologous region of *MYB* genes in Rutaceae plants, we expect specific bands to appear in plants of the same family. On the other hand, we also expect to produce polymorphic bands for the identification of different species and origins with *MYB* gene family markers. Different species of plants are biological groups with certain physiological and morphological characteristics, and there is a phenomenon of reproductive isolation between species (Wendel et al., 2016). Therefore, different species of plants

Table 4
Statistical results of MYB primers on identification of different origins.

Primer names	Total no. of bands	No. of polymorphic bands	Percentage ofpolymorphism (%)	PIC	Rp
M1	15	15	100.00	0.903 5	7.250 0
M2	12	5	41.67	0.885 7	2.000 0
M3	13	11	84.62	0.870 7	3.500 0
M4	15	12	80.00	0.904 5	4.500 0
M5	13	13	100.00	0.856 1	4.250 0
M6	12	11	91.67	0.847 7	4.250 0
M7	11	9	81.82	0.850 4	3.5000
M8	13	11	84.62	0.869 6	3.5000
M9	13	11	84.62	0.860 1	4.000 0
M10	17	13	76.47	0.903 8	5.000 0
Average	13.4	11.1	82.84	–	–
Total	134	111	–	–	–

have different degrees of variation in genotypes. The growth environment of plants in different producing areas is different. In different growth environments, plants have evolved a series of gene regulation mechanisms that adapt to the environment (Amtmann, Bohnert, & Bressan, 2005). Therefore, *MYB* gene family polymorphic markers have the potential to distinguish different species and origins of plants. *MYB* gene family as functional genes have a wide range of biological functions in plants. They are involved in many life processes in plants, including regulating the growth and development of plants, participating in cell morphology and morphogenesis, regulating primary and secondary metabolic reactions, and responding to biotic and abiotic adverse environmental stress, etc (Erpen, Devi, Grosser, & Dutt, 2018). Therefore, the *MYB* gene family polymorphism markers can sequence the label bands to explore more undeveloped functions of *MYB* genes in plants.

4.2. MYB is a potentially novel type of polymorphic molecular marker

In this study, the design concept of MYB molecular marker was based on the principle of SCoT molecular markers. Studies on the authenticity of CRP can be conducted not only from the medicinal materials, but also in combination with the corresponding geographical environmental factors. MYB transcription factor could regulate the growth and development of plants, respond to the biotic and abiotic adverse environmental stresses, and regulate the effects of primary and secondary metabolites. Therefore, *MYB* gene family polymorphism was feasible to study the authenticity of CRPs. According to the highly conserved region of MYB domain of *Citrus* plants, 10 primers with clear bands were screened out from 21 primers initially designed. Then, MYB primers were screened for polymorphism in different families, genera and species. The results showed that nine MYB primers had the ability to distinguish plant relationships. Then, we screened the polymorphism of CRPs from different places and studied their local connotation. Seven MYB primers could distinguish Xinhui CRP from other places, and the primers M1 and M10 had the best identification effect, and eight different places could be distinguished from each other. The innovative *MYB* gene family polymorphism markers with rich polymorphism and good reproducibility can realize the authentic identification of Xinhui CRP.

4.3. Novel molecular markers development is significant for CRP identification and conservation

The *MYB* gene family polymorphism markers developed based on SCoT molecular markers can be used to study the authenticity of Xinhui CRP, and can distinguish Xinhui CRP from other native CRPs, providing a certain reference for the authenticity study. And the identification methods of ancient and modern authentic producing origins, not only reflect the cultural characteristics of traditional Chinese medicine, the need to ensure the sustainable development of traditional Chinese medicine, but also reveal the modern scientific connotation of traditional Chinese medicine. It will provide a basis for authenticity identification, resource protection, product development and market guidance of CRPs. In this study, *MYB* gene family polymorphism markers were used to study the authenticity of Xinhui CRPs. The results showed that the genetic distance of CRPs in different regions was far. It can also be combined with downstream technology, such as the comparison of gene transcription level and protein level, in order to study the scientific connotation of authentic medicinal materials. With the development of science and technology, further interdisciplinary research such as chemistry, pharmacology, genetic breeding and molecular biology should be carried out to further clarify the molecular connotation of traditional Chinese medicine, and there will be broad space for development in the future.

5. Conclusion

In this study, *MYB* gene family polymorphism marker primers were designed to identify CRP by identifying plants of different families, genera, and species, but only primers M6 and M7 could not identify plants of different species of *Citrus*. Primers M3, M4, M5, M6, M7, M8, M9 could distinguish Xinhui CRP, the geoherb of CRP, from other origins. Primers M1 and M10 could distinguish eight different origins from each other. So, we anticipate that the *MYB* gene family polymorphism markers have great potential. The main applications are genetic polymorphism analysis and identification including species, varieties, and regions. Furthermore, based on the rich physiological functions of the *MYB* gene family, sequencing the label DNA can explore more undeveloped functions of *MYB* genes in plants. In summary, a novel DNA marker technique that designed primers based on *MYB* gene family was developed. It is agarose-based, so it is simple and relatively cheap to use. Furthermore, this molecular marker we found can distinguish CRP from different origins successfully, so it may also have great application potential and prospect in the identification of CRP from different origin and even other traditional Chinese medicine. With the continuous development of new technologies such as genomics, bioinformatics, and gene chips, as well as their integration and mutual penetration with molecular marker technology, DNA molecular marker technology will have broader development space.

CRediT authorship contribution statement

Qiqing Cheng: Methodology, Formal analysis, Investigation, Validation, Visualization, Writing – review & editing. **Ziyu Tang:** Methodology, Data curation, Formal analysis, Investigation, Validation, Visualization. **Yue Ouyang:** Methodology, Data curation, Formal analysis, Investigation, Validation, Visualization. **Chunsong Cheng:** Methodology, Investigation, Validation, Visualization. **Chi-**

chou Lao: Methodology, Data curation, Formal analysis, Investigation, Validation, Visualization. **Hao Cui:** Methodology, Data curation, Formal analysis, Investigation, Validation, Visualization. **Hua Zhou:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing. **Yongshu Liang:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chmed.2025.02.006.

References

Amtmann, A., Bohnert, H. J., & Bressan, R. A. (2005). Abiotic stress and plant genome evolution. *Search for new models. Plant Physiology, 138*(1), 127–130.
Arnold, K., Bordoli, L., Kopp, J., & Schwede, T. (2006). The SWISS-MODEL workspace: A web-based environment for protein structure homology modelling. *Bioinformatics, 22*(2), 195–201.
Bhattacharyya, P., Kumaria, S., Kumar, S., & Tandon, P. (2013). Start *Codon* Targeted (SCoT) marker reveals genetic diversity of *Dendrobium nobile* Lindl., an endangered medicinal orchid species. *Gene, 529*(1), 21–26.
Biedenkapp, H., Borgmeyer, U., Sippel, A. E., & Klempnauer, K. H. (1988). Viral myb oncogene encodes a sequence-specific DNA-binding activity. *Nature, 335*(6193), 835–837.
Biswas, M. K., Baig, M. N. R., Cheng, Y. J., & Deng, X. X. (2010). Retro-transposon based genetic similarity within the genus *Citrus* and its relatives. *Genetic Resources and Crop Evolution, 57*(7), 963–972.
Blair, M. W., Panaud, O., & McCouch, S. R. (1999). Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics, 98*(5), 780–792.
Bustamante, K., Santos-Ordóñez, E., Miranda, M., Pacheco, R., Gutiérrez, Y., & Scull, R. (2019). Morphological and molecular barcode analysis of the medicinal tree *Mimusops coriacea* (A.DC.) Miq. collected in Ecuador. *PeerJ, 7*, e7789.
Collard, B. C. Y., & MacKill, D. J. (2009). Start *Codon* targeted (SCoT) polymorphism: A simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant Molecular Biology Reporter, 27*(1), 86–93.
Dubos, C., Stracke, R., Grotewold, E., Weisshaar, B., Martin, C., & Lepiniec, L. (2010). MYB transcription factors in *Arabidopsis*. *Trends in Plant Science, 15*(10), 573–581.
Erpen, L., Devi, H. S., Grosser, J. W., & Dutt, M. (2018). Potential use of the DREB/ERF, MYB, NAC and WRKY transcription factors to improve abiotic and biotic stress in transgenic plants. *Plant Cell, Tissue and Organ Culture (PCTOC), 132*(1), 1–25.
Ghaffarian, S., & Mohammadi, S. A. (2023). Genetic diversity and population structure of *Melissa officinalis* L. from Iran as reveled by retrotransposon insertional polymorphism. *Genetic Resources and Crop Evolution, 70*(8), 2521–2532.
Han, K., Wang, M., Zhang, L., & Wang, C. (2018). Application of molecular methods in the identification of ingredients in Chinese herbal medicines. *Molecules, 23*(10), 2728.
He, J., Xu, Y., Huang, D., Fu, J., Liu, Z., Wang, L., ... Xu, Q. (2022). TRIPTYCHON-LIKE regulates aspects of both fruit flavor and color in *Citrus*. *Journal of Experimental Botany, 73*(11), 3610–3624.
Horibata, A., & Kato, T. (2020). Phylogenetic relationships among accessions in *Citrus* and related genera based on the insertion polymorphism of the *CIRE1* retrotransposon. *Open Agriculture, 5*(1), 243–251.
Huang, D., Tang, Z., Fu, J., Yuan, Y., Deng, X., & Xu, Q. (2020). CsMYB3 and CsRuby1 form an 'activator-and-repressor' loop for the regulation of anthocyanin biosynthesis in *Citrus*. *Plant & Cell Physiology, 61*(2), 318–330.
Huang, Y. J., Zhou, Q., Huang, J. Q., Zeng, Y. R., Wang, Z. J., Zhang, Q. X., ... Zheng, B. S. (2015). Transcriptional profiling by DDRT-*PCR* analysis reveals gene expression during seed development in *Carya cathayensis* Sarg. *Plant Physiology and Biochemistry, 91*, 28–35.
Kiefer, F., Arnold, K., Künzli, M., Bordoli, L., & Schwede, T. (2009). The SWISS-MODEL Repository and associated resources. *Nucleic Acids Research, 37*(Suppl 1), D387–D392.
Li, S. Z., Zeng, S. L., Wu, Y., Zheng, G. D., Chu, C., Yin, Q., ... Liu, E. H. (2019). Cultivar differentiation of Citri Reticulatae Pericarpium by a combination of hierarchical three-step filtering metabolomics analysis, DNA barcoding and electronic nose. *Analytica Chimica Acta, 1056*, 62–69.
Liu, C., Wang, X., Xu, Y., Deng, X., & Xu, Q. (2014). Genome-wide analysis of the R2R3-*MYB* transcription factor gene family in sweet orange (*Citrus sinensis*). *Molecular Biology Reports, 41*(10), 6769–6785.
Long, W., Hu, Z., Wei, L., Chen, H., Liu, T., Wang, S., ... Fu, H. (2022). Accurate identification of the geographical origins of lily using near-infrared spectroscopy combined with carbon dot-tetramethoxyporphyrin nanocomposite and chemometrics. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 271*, 120932.
Mahjbi, A., Baraket, G., Oueslati, A., & Salhi-Hannachi, A. (2015). Start Codon Targeted (SCoT) markers provide new insights into the genetic diversity analysis and characterization of Tunisian *Citrus* species. *Biochemical Systematics and Ecology, 61*, 390–398.
Mishra, P., Kumar, A., Nagireddy, A., Mani, D. N., Shukla, A. K., Tiwari, R., & Sundaresan, V. (2016). DNA barcoding: An efficient tool to overcome authentication challenges in the herbal market. *Plant Biotechnology Journal, 14*(1), 8–21.
Nadeem, M. A., Nawaz, M. A., Shahid, M. Q., Doğan, Y., Comertpay, G., Yıldız, M., ... Baloch, F. S. (2018). DNA molecular markers in plant breeding: Current status and recent advancements in genomic selection and genome editing. *Biotechnology & Biotechnological Equipment, 32*(2), 261–285.
Porebski, S., Bailey, L. G., & Baum, B. R. (1997). Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter, 15*(1), 8–15.
Rai, M. K. (2023). Start codon targeted (SCoT) polymorphism marker in plant genome analysis: Current status and prospects. *Planta, 257*(2), 34.
Roy, N. S., Choi, J. Y., Lee, S. I., & Kim, N. S. (2015). Marker utility of transposable elements for plant genetics, breeding, and ecology: A review. *Genes & Genomics, 37*(2), 141–151.
Song, L., Xiong, P., Zhang, W., Hu, H., Tang, S., Jia, B., & Huang, W. (2022). Mechanism of *Citri Reticulatae Pericarpium* as an anticancer agent from the perspective of flavonoids: A review. *Molecules, 27*(17), 5622.
Thakur, S., & Vasudev, P. G. (2022). MYB transcription factors and their role in medicinal plants. *Molecular Biology Reports, 49*(11), 10995–11008.
Wendel, J. F., Jackson, S. A., Meyers, B. C., & Wing, R. A. (2016). Evolution of plant genome architecture. *Genome Biology, 17*, 37.
Yin, L., Zhou, J., Chen, D., Han, T., Zheng, B., Younis, A., & Shao, Q. (2019). A review of the application of near-infrared spectroscopy to rare traditional Chinese medicine. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 221*, 117208.
Yu, X., Sun, S., Guo, Y., Liu, Y., Yang, D., Li, G., & Lü, S. (2018). *Citri Reticulatae Pericarpium* (Chenpi): Botany, ethnopharmacology, phytochemistry, and pharmacology of a frequently used traditional Chinese medicine. *Journal of Ethnopharmacology, 220*, 265–282.
Zhang, P., Liu, X., Yu, X., Wang, F., Long, J., Shen, W., ... Zhao, X. (2020). The MYB transcription factor CiMYB42 regulates limonoids biosynthesis in *Citrus*. *BMC Plant Biology, 20*(1), 254.
Zhang, Q., Xue, R., Mei, X., Su, L., Zhang, W., Li, Y., ... Lu, T. (2024). A study of volatiles of young *Citrus* fruits from four areas based on GC-MS and flash GC e-nose combined with multivariate algorithms. *Food Research International, 177*, 113874.
Zheng, G., Chao, Y., Luo, M., Xie, B., Zhang, D., Hu, P., Yang, X., ... Wei, M. (2020). Construction and chemical profile on "activity fingerprint" of *Citri Reticulatae Pericarpium* from different cultivars based on HPLC-UV, LC/MS-IT-TOF, and principal component analysis. *Evidence-Based Complementary and Alternative Medicine, 2020*, 4736152.
Zhu, F., Luo, T., Liu, C., Wang, Y., Yang, H., Yang, W., ... Cheng, Y. (2017). An R2R3-MYB transcription factor represses the transformation of α- and β-branch carotenoids by negatively regulating expression of CrBCH2 and CrNCED5 in flavedo of *Citrus* reticulate. *New Phytologist, 216*(1), 178–192.
Zou, J., Wang, J., Ye, W., Lu, J., Li, C., Zhang, D., ... Liu, Z. (2022). *Citri Reticulatae Pericarpium* (Chenpi): A multi-efficacy pericarp in treating cardiovascular diseases. *Biomedicine & Pharmacotherapy, 154*, 113626.