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Identification of AFLP fragments linked to hydroxysafflor yellow A in Flos Carthami and conversion to a SCAR marker for rapid selection

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Abstract Hydroxysafflor yellow A (HSYA), an important active compound in treating focal cardiac and cerebral ischemia, is uniquely present in flower petals of *Carthamus tinctorius.* In this study, inheritance and molecular marker analyses for HSYA trait in safflower were carried out. HSYA contents in parents, cross hybridized F_1 and F_2 individuals were analyzed by high performance liquid chromatography. Results revealed that the presence/absence of HSYA was controlled by one major nuclear gene termed *HSya*. A total of 48 AFLP primer combinations were screened, and bulked segregant analysis was performed by preparing two pools of 10 present-HSYA and ten absent-HSYA plants selected from the 498 individuals of the F_2 segregating population. Four AFLP markers, AFLP-5, AFLP-7, AFLP-15 and AFLP-16, were identified to be closely associated with *HSya*. Of those, AFLP-16 was the closest to *HSya*, estimated at about 9.4 cM in genetic distance. The dominant AFLP-16 marker was converted into a simple sequence characterized amplified region marker based on the sequence information of the cloned flanking regions of the AFLP fragment and was designated as SCM16. Our result has direct application for marker-assisted selection of quality breeding in safflower.

Keywords *Carthamus tinctorius* L. · Bulked segregant analysis (BSA) · AFLP · Hydroxysafflor yellow A (HSYA) · SCAR (sequence characterized amplified region)

Abbreviations

BSA	Bulked sergeant analysis
AFLP	Amplified fragment length polymorphism
HSYA	Hydroxysafflor yellow A

Introduction

Flos Carthami, the dried flower petal of safflower (*Carthamus tinctorius* L.), is an important crude drug in traditional Chinese medicine for promoting blood circulation and removing obstruction in the channels (Yang et al. 2004). It has long been used clinically in China for the prevention and treatment of cardiovascular and cerebrovascular diseases, including cerebral

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thrombus, cerebral embolism, cerebral ischemia and lacuna embolism (Bie 2003; Jiang et al. 2005; Siow et al. 2000; Liu et al. 1997; Luo et al. 2004). Many studies show that chemical constituents in Flos Carthami mainly include flavanoids, indoles, lignanoids, alkyldiol, organic acids and sterols (Li and Chen 1998; Masao et al. 1992; Obara and Onodera 1979; Onodera et al. 1981, 1989; Zhou et al. 2006).

Hydroxysafflor yellow A (HSYA) is one of the main active components in Flos Carthami and its structure was first reported in 1993 (Meselhy et a1. 1993). (Two new quinoch alcone yellow pigment from *C. tinctorius* and Ca^{2+} antagonistic activity of Tinctormine. Chem Pharm Bull 4l 1796.) Many studies have demonstrated that HSYA is a good potential agent for the protection and treatment of focal cardiac and cerebral ischemia (Sato et al. 2005; Jin et al. 2004; Zhang et al. 2005), and the underlying mechanism is supposed to be associated with its inhibitory effects on the thrombogenesis and platelet aggregation and with its beneficial action on regulation of PGI2/TXA2 and blood rheological changes in rats (Hai et al. 2005). HSYA is found to increase hypoxia tolerance significantly, dilate the coronary artery and promote coronary blood flow, and inhibit ADP-induced platelet aggregation in rabbits (Zhu et al. 2005). Therefore, HSYA in Flos Carthami is an economically important trait for safflower breeding and medicinal production.

To date, the inheritance and molecular marker for the HSYA trait of safflower have not been reported. In this present study, we focused on genetic analysis and development of a molecular marker linked to this unique compound in safflower. We found that HSYA is genetically controlled by one dominant nuclear gene termed as *HSya*. Moreover, we studied the *HSya*-related gene fragment with bulk segregate analysis (BSA) (Michelmore et al. 1991) by using the optimized safflower amplified fragment length polymorphisms (AFLP) reaction system (Zhang et al. 2006). Four DNA fragments linked to the *HSya* and designated as AFLP-5, AFLP-7, AFLP-15 and AFLP-16, were identified, respectively. One of the AFLP markers closely linked to HSYA trait was also converted into a SCAR marker. The conversion of a dominant AFLP marker into a simple, SCAR marker has direct application for marker-assisted selection (MAS) in safflower breeding. The following is our first report of the study.

Materials and methods

Plant material

Two parental strains (No. 0016 and No. 0025) of what were selected from the Chinese populations by our laboratory, HSYA was found in the former (P_1) with a content of 2.11% ± 0.09% (n = 83), while HSYA was not found in the latter (P_2) with a content of 0.00% ± 0.00% (n = 89). The reciprocal crosses ($P_1 \times P_2$, $P_2 \times P_1$) were made, resulting in 87, 93 F_1 seeds, artificially by hand, in the summer of 2003 at Medicinal Plant Garden of Second Military Medical University, Shanghai, China. The F_2 seeds of the crosses were produced in field of Sanya, Hainan province, by bagging F_1 plants in paper bags prior to florescence during 2003 and 2004. A segregating F_2 population was obtained by sifting a single F_1 plant in 2004 and 2005 in the garden mentioned. Four hundred and ninety-eight segregating F_2 individuals were obtained.

Determination of HSYA content by HPLC in *C. tinctorius* L.

HSYA standard sample ($C_{27}H_{32}O_{16}$) was extracted from Flos Carthami in our laboratory. Its purity was estimated at 99.5% by HPLC analysis and its structure was shown in Fig. 1 (Guo et al. 2006). Chromatography was performed with Agilent1100 (USA) Model 510 binary gradient equipment; an Agilent1100 chromatography workstation was equipped with an injection valve with 20-μl sample loop. HSYA was separated on a 250 × 4.6 mm (i.d.), 5 μm particle, ZORBAX SB-C_{18} column (Agilent Company). Optimum HPLC separation was achieved by use of 10% aqueous acetonitrile at a 1.0 ml/min low rate. Detection wavelength was 403 nm and temperature was 22°C. Dry safflower florets (~0.5 g) were weighed accurately into a 250 ml tube, and HSYA was

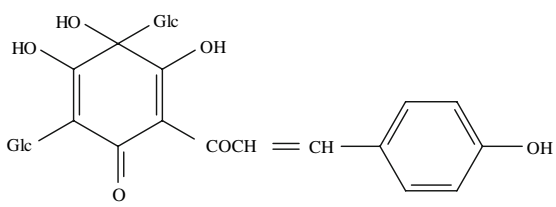

Fig 1 The formula of HSYA ($C_{27}H_{32}O_{16}$)

extracted with 100 ml water by soaking overnight, sonicated for 20 min in a sealed, and filtered through a 0.45 ml Nylon syringe filter (Millex-HN, Ireland) before injection for HPLC analysis.

AFLP and BSA

DNA was extracted from safflower plants using a modified CTAB method (Zhang et al. 2006). AFLP fingerprints were generated based on the protocol described by Vos et al. (1995) with minor modifications. All reagents required for AFLP analysis were obtained from NEB Company. PCR reactions were performed in a Biometra T gradient PCR thermal cycler. Genomic DNA (250 ng) was restricted with *Eco*RI and *Mse*I (2.5 U each) in restriction buffer (50 mM Tris–HCL, Ph 7.5, 50 mM Mg-acetate, 250 mM K-acetate) in a final volume of 25 µl. *Eco*RI and *Mse*I adapters were subsequently ligated to the digested DNA fragments. The adapter-ligated DNA was pre-amplified with AFLP primers, each having one selective nucleotide using the following cycling parameters: 20 cycles of 30 s at 94°C, 60 s at 56°C and 60 s at 72°C. The pre-amplified DNA was diluted (1:50), and an aliquot was used for selective amplification with the *Eco*RI and *Mse*I primers having three selective nucleotides at the 3′ ends. The following cycling parameters were used for selective amplification: 1 cycle of 30 s at 94°C, 30 s at 56°C and 60 s at 72°C. The annealing temperature was then lowered by 0.7°C per cycle during the first 12 cycles, and then 23 cycles were performed at 94°C for 30 s, 56°C for 30 s and 72°C for 60 s. The reaction products were resolved in 6% polyacrylamide gels and silver-staining. For BSA–AFLP analysis, DNA of 10 present- and 10 absent-HSYA individuals with contents $\geq$2.00% and 0.00% were randomly selected from 498 F_2 progenies and bulked into two pools.

Data analysis

To determine the inheritance of the HSYA trait, the χ^2-test was applied to evaluate the goodness-of-fit for F_2 data based on a gene segregation model. Linkage analysis for AFLP markers was performed using the 213 plants selected randomly from the F_2 segregating population and the program MapMaker V3.0b (Lander et al. 1987).

Cloning of AFLP markers

The 11 AFLP fragments (Table 2) amplified specifically for either presence or absence of bulks were cloned for analysis. These AFLP bands were excised from dried polyacrylamide gels, re-hydrated in TE for 1 h at room temperature and transferred to a 500 µl elution buffer (0.5 MNH4AC, 10 mM magnesium acetate, 1 mM EDTA, pH 8.0, 0.1% SDS) at 37°C. 1.0 µl supernatant was used as template for PCR amplification using primers and reaction conditions similar to those used for AFLP reaction. The amplified products were electrophoresed in 1% agarose gel. The bands were excised from the gel, and the DNA was cloned into the plasmid vector PMD18D (Takara Company). Recombinant plasmid DNA was isolated and sequenced using the dideoxy method.

Conversion of AFLP marker into SCAR marker

Based on the sequence analysis of the cloned fragments obtained after AFLP analysis, the SCAR primers were designed and synthesized. Sequences of this pair of SCAR primers are as follows: SCM16 primer 1 (Forward): 5′-GACTGCGTACCAATTCACT-3′; SC M16 primer 2 (Reverse): 3′-GATGAAGTCCTGAGT AACAG-5′. The PCR-amplified method was adopted to genomic DNA from present- and absent- HSYA individual lines by using primer pair SCM16. Genomic DNA (50-ng aliquots) was used in a standard PCR reaction containing 2.5 mM $MgCl_2$, 2.0 mM dNTPs and 0.5 U *Taq* polymerase. PCR conditions for amplification were 94°C for 3 min, followed by 40 cycles of 94°C for 45 s, 69°C for 45 s, 71°C for 1.5 min. The PCR products were visualized after agarose gel eletrophoresis.

Results

Inheritance of present- and absent-HSYA in safflower

In reciprocal crosses of two parental strains, HSYA were found in all F_1 individuals. The content of HSYA was 1.97% $\pm$ 0.13% and 2.03% $\pm$ 0.17%, respectively. Therefore, the presence of HSYA was considered dominant over the absence of HSYA. In

Table 1 Content and classification of the plants in the F_1 and F_2 generations for present- and absent- HSYA

Generation	Present-HSYA		Absent-HSYA		Expected ratio	χ^2	*P*
	Content (Mean ± SD, %)	Number of plants	Content (Mean ± SD, %)	Number of plants			
$F_1(P_1 \times P_2)$	1.97 ± 0.13	87	0.00 ± 0.00	0	–	–	–
$F_1(P_2 \times P_1)$	2.03 ± 0.17	93	0.00 ± 0.00	0	–	–	–
F_2	1.65 ± 0.51	372	0.00 ± 0.00	126	3:1	0.011	0.900

Table 2 AFLP fragments specific to the bulks

Primer designation	Primer combination	AFLP Marker designation	Approximate size of marker (bp)	Specificity of HSYA
AF26356	E-CAT + M-AGC	AFLP-1	750	Present
AF26356	E-CAT + M-AGC	AFLP-3	550	Absent
AF26356	E-CAT + M-AGC	AFLP-4	335	Absent
AF26361	E-ACT + M-CAG	AFLP-5	936	Present
AF26361	E-ACT + M-CAG	AFLP-7	852	Present
AF26363	E-AAC + M-AGG	AFLP-9	550	Present
AF26363	E-AAC + M-AGG	AFLP-10	350	Present
AF26363	E-AAC + M-AGG	AFLP-12	250	Present
AF26363	E-AAC + M-AGG	AFLP-14	150	Absent
AF26386	E-ACC + M-CTC	AFLP-15	730	Present
AF26386	E-ACC + M-CTC	AFLP-16	277	Present

the F_2 generation, individuals were classified in two distinct groups confirming to the presence and absence ratio of 3:1 (the presence type had a content of 1.65% ± 0.51%, whereas the absence type had a content of 0.00% ± 0.00%) (Table 1). Therefore, it implied that HSYA trait was controlled by a single major nuclear gene with two alleles, in which *HSya* was completely dominant over *hsya* (the allele for absence).

AFLP markers linked to gene *HSya*

To identify markers linked to gene *HSya*, a total of 48 primer combinations were used to test DNA from the parents No. 0016 and No. 0025. Of these, 16 primer pairs (date not shown) revealed polymorphisms between parents were selected for BSA. The primers were amplified ~40–70 bands per assay. A total of 769 bands were obtained from the six different primer combinations and revealed 16–43% polymorphism between the parents. AFLP bands present in one pool and absent in the other were regarded as candidate markers. Four primer combinations produced DNA fragments present only in one pool and absent in the other. Eleven AFLP bands specific either to the present- and absent-HSYA pool were identified (Table 2).

AFLP linkage map

To confirm the linkage of candidate AFLP markers to the *HSya* locus, 213 segregating F_2 individuals were screened for polymorphism. Figures 2 and 3 show a representative amplification profile with two different primer combinations. With the primer combination *Eco*RI-ACT-*Mse*I-CAG two bands at 936 bp and 852 bp (indicated by *arrows* in Fig. 2) were almost present in the present-HSYA parent No. 0016 and individuals which were present-HSYA. They were designated as AFLP-5 and AFLP-7 (Fig. 2). These bands were not present in the absent-HSYA parent No.0025 and absent-HSYA segregating individuals. With the primer *combination* of *Eco*RI-ACC-*Mse*I-CTC, a fragment

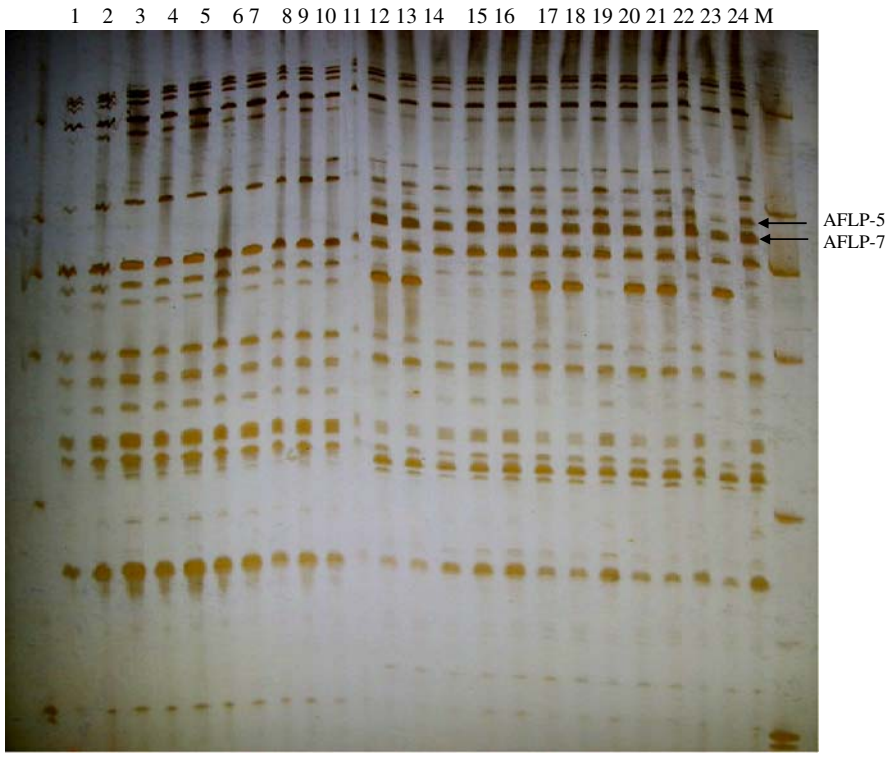

Fig. 2 A portion of the AFLP Silver-staining generated with primer combinations *Eco*RI-ACT-*Mse*I-CAG for *Carthamus tinctorius* L. DNA. 1–9 Segregating individuals of absent-HSYA. 10 ♂, No. 0025 Parent of absent-HSYA. 11 Bulk of absent-HSYA. 12 Bulk of present-HSYA. 13 ♀, No.0016 Parent of present-HSYA. 14–24 Segregating individuals of present-HSYA. M DL 2000 Marker. The AFLP markers linked to presence of HSYA are indicated by *arrows*

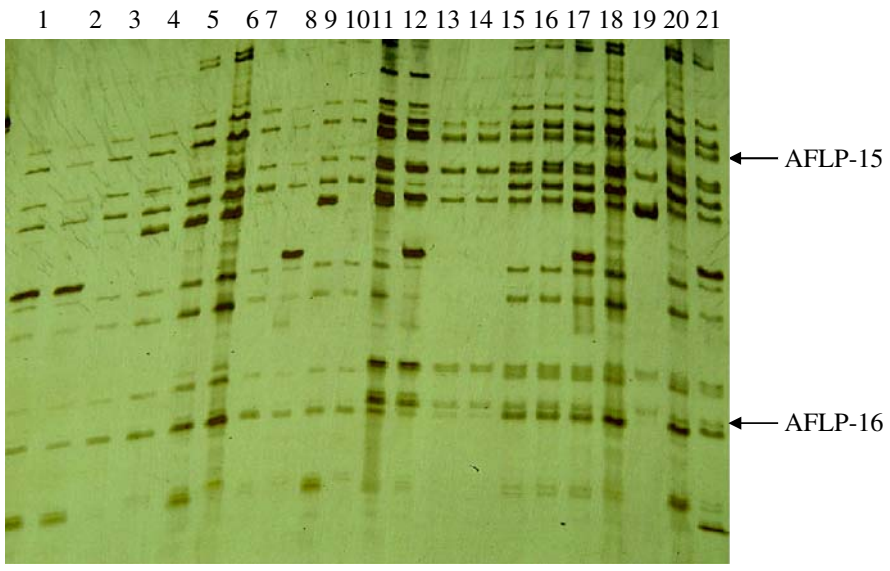

Fig. 3 A portion of the AFLP Silver-staining generated with primer combinations *Eco*RI-ACC-*Mse*I-CTC for *Carthamus tinctorius* L. DNA. 1–8 Segregating individuals of absent-HSYA. 9 ♂, No. 0025 Parent of absent-HSYA. 10 Bulk of absent-HSYA. 11 Bulk of present-HSYA. 12 ♀, No. 0016 Parent of present-HSYA. 13–21 Segregating individuals of present-HSYA. The AFLP markers linked to present-HSYA are indicated by *arrows*

of 730 bp and a fragment of 277 bp (indicated by *arrows* in Fig. 3) were almost present in the present-HSYA parent No. 0016 and individual present-HSYA ones. They were designated as AFLP-15 and AFLP-16. Evaluated with the program MapMaker V3.0b, the genetic distances of AFLP-7, AFLP-5, AFLP-15 and AFLP-16 from the *HSya* locus were 14.2, 11.8, 9.9 and 9.4 cM(Fig. 4), respectively. The sequence data of these fragments were as follows:

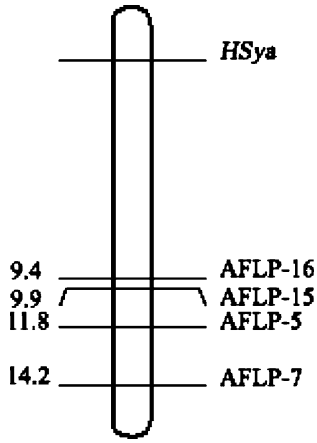

Fig. 4 Local linkage map around the *HSya* locus consisting of four AFLP markers. *Left* map distances (Kosambi, cM); *right* names of AFLP markers

AFLP-7

AGACTGCGTACCAATTCACCGCGTACTAATT
CACTTACTCTTTTTATGGATTTGAAATATAG
CACATCCTGGAGAGGTAAGAGATAGAGGAA
CATTCCAACTGCTTAGTTGGTTAGGGGTGTT
ACTCCAGGACTCATCAGCTTCCTTCCAAANG
GGGAGAAGAGGAAAGCAAAGAAGATTGTG
AAAGAAGCTATAGACTGCGTACCAATTCAC
CGCGTACTAATTCACTTACTCTTTTTATGGA
TTTGAAATATAGCACATCCTGGAGAGGTAA
GAGATAGAGGAAC AGACTGCGTACCAATTC
ACCGCGTACTAATTCACTTACTCTTTTTATG
GATTTGAAATATAGCACATCCTGGAGAGGT
AAGAGATAGAGGAACATTCCAACTGCTTAG
TTGGTTAGGGGTGTTACTCCAGGACTCATCA
GCTTCCTTCCAAANGGGGAGAAGAGGAAAG
CAAAGAAGATTGTGAAAGAAGCTATAGACT
GCGTACCAATTCACCGCGTACTAATTCACTT
ACTCTTTTTATGGATTTGAAATATAGCACAT
CCTGGAGAGGTAAGAGATAGAGGAAC AGA
CTGCGTACCAATTCACCGCGTACTAATTCAC
TTACTCTTTTTATGGATTTGAAATATAGCAC
ATCCTGGAGAGGTAAGAGATAGAGGAACAT
TCCAACTGCTTAGTTGGTTAGGGGTGTTACT
CCAGGACTCATCAGCTTCCTTCCAAANGGG
GAGAAGAGGAAAGCAAAGAAGATTGTGAA
AGAAGCTATAGACTGCGTACCAATTCACCG
CGTACTAATTCACTTACTCTTTTTATGGATT
TGAAATATAGCACATCCTGGAGAGG

AFLP-5

GACTGCGTACCAATTCACCTACTAATTCACTT
TGATCATATTTCTGAGAATAATCTACTGGCAG
GGGATAGAGTAATAACAGATCCTCTTGCAAG
CCTTCAGCATGGAAGCGGAAAATAAAAGATA
TAATATGGATTGCGCCAAGTGGTGGCAGGGA
CAGGCCAGCTCCTCGATGTTTGTTCTGTGTGA
ACCTGACTGCGTACCAATTCACCTACTAATTC
ACTTTGATCATATTTCTGAGAATAATCTACTG
GCAGGGGATAGAGTAATAACAGATCCTCTTG
CAGACTGCGTACCAATTCACCTACTAATTCAC
TTTGATCATATTTCTGAGAATAATCTACTGGC
AGGGGATAGAGTAATAACAGATCCTCTTGCA
AGCCTTCAGCATGGAAGCGGAAAATAAAAGA
TATAATATGGATTGCGCCAAGTGGTGGCAGG
GACAGGCCAGCTCCTCGATGTTTGTTCTGTGT
GAACCTGACTGCGTACCAATTCACCTACTAAT
TCACTTTGATCATATTTCTGAGAATAATCTAC
TGGCAGGGGATAGAGTAATAACAGATCCTCT
TGCAGACTGCGTACCAATTCACCTACTAATTC
ACTTTGATCATATTTCTGAGAATAATCTACTG
GCAGGGGATAGAGTAATAACAGATCCTCTTG
CAAGCCTTCAGCATGGAAGCGGAAAATAAAA
GATATAATATGGATTGCGCCAAGTGGTGGCA
GGGACAGGCCAGCTCCTCGATGTTTGTTCTGT
GTGAACCTGACTGCGTACCAATTCACCTACTA
ATTCACTTTGATCATATTTCTGAGAATAATCT
ACTGGCAGGGGATAGAGTAATAACAGATCCT
CTTGCAGACTGCGTACCAATTCACCTACTAAT
TCACTTTGATCATATTTCTGAGAATAATCTAC
TGGCAGGG

AFLP-15

GACTGCGTACCAATTCACCTGCGTACCAATT
CACCCGTGCACCCAGTCCATGCTGCTTTAGC
TTTTTTGATCGCGCGGATTTCCTTTATCCCCT
ATTTTTCGAAAAGCTTTCAAAACAAAATGCT
ACTCTTTCGAAATGGGGGCTACGTATTTAGC
TAGGCTAAATCGTGGGGCTAGCTAGGCCTA
GCTATTTGACTGCGTACCAATTCACCTGCGT
ACCAATTCACCCGTGCACCCAGTCCATGCTG
CTTTAGCTTTTTTGATCGCGCGGATTTCCTTT
ATCCCCGACTGCGTACCAATTCACCTGCGT
ACCAATTCACCCGTGCACCCAGTCCATGCTG
CTTTAGCTTTTTTGATCGCGCGGATTTCCTTT
ATCCCCTATTTTTCGAAAAGCTTTCAAAACA
AAATGCTACTCTTTCGAAATGGGGGCTACG

TATTTAGCTAGGCTAAATCGTGGGGCTAGCT
AGGCCTAGCTATTTGACTGCGTACCAATTCA
CCTGCGTACCAATTCACCCGTGCACCCAGT
CCATGCTGCTTTAGCTTTTTTGATCGCGCGG
ATTTCCTTTATCCCCGACTGCGTACCAATTC
ACCTGCGTACCAATTCACCCGTGCACCCAG
TCCATGCTGCTTTAGCTTTTTTGATCGCGCG
GATTTCCTTTATCCCCTATTTTTCGAAAAGC
GATGAGTCCTGAGTAACTCCTTGATGAAGT
CCTGAG TAACTC

AFLP-16

GACTGCGTACCAATTCACCAAGTCCATGTC
AGACGCCGATCCATTAGCTTGAAGGTGATG
CAATACAGAAGGTCAAATGCCGATTCCTGT
CGGGGTACATGGGGAGAATAAATATAAAAC
AAGGCACCTCCTACACTACTTCTAATAAATA
AATCGGGTCTTAGTCTAACTGCATCTGACAA
GACTGAACTTCGACTGCGTACCAATTCACC
AAGTCCATGTCAGACGCCGATCCATTAGCTT
GAAGGTGATGCAATACAGAAGGTCAAATGC
CG

Amplification of SCAR from genomic DNA

It was necessary to convert the AFLP markers into simple PCR-based markers for safflower breeding programs. Based on the flanking sequence of AFLP-16 marker, which was most closely linked to the *HSya* locus of the four obtained fragments, primers were designed for direct amplification of genomic DNA as described in the "Materials and methods." Primers were used to amplify DNA from the two parents and 213 F_2 segregation. The maternal plant and present-HSYA F_2 individuals produced a strong band at 298 bp, whereas the paternal plant and absent-HSYA plants produced no bands, whose banding patterns were similar to those of AFLP-16 markers (partly refer to Fig. 5). Thus, a dominant AFLP-16 marker was converted into a co-dominant SCAR marker and was subsequently designated as SCM16. By initial verification, SCM16 showed good specificity and stability, therefore it could be applied to the molecular MAS for HSYA trait.

Discussion and conclusion

Our previous study and many other studies have showed that flavonoids are the main chemical constituents of Flos Carthami (Kim et al. 1992; Hattori et al. 1992; Zhang et al. 2002, 2005), of which HSYA plays a key role in effects of Flos Carthami against cardiovascular and cerebral ischemia induced injuries. In our study, we first used a combination of BSA and AFLP techniques to identify markers linked to the present HSYA in safflower. The parents obtained in our laboratory through long systematic screening and purification included a strain (No. 0016) stable in HSYA content and a strain (No. 0025) stable in absent-HSYA. Our study revealed that HSYA trait was controlled by one major nuclear gene with two alleles, in which *HSya*, was completely dominant over *hsya*(the allele for absence). A high percentage of polymorphism (17–44%) was observed between these two parents with AFLP. Between the bulks of F_2 offspring by BSA, 11 markers were polymorphic, and four co-segregated with present HSYA

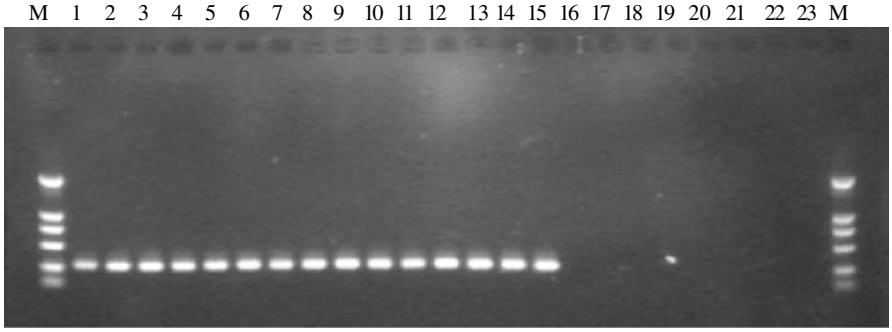

Fig. 5 Specific amplified result with SCAR primers. 1–13: Segregating individuals of present-HSYA.14 The bulk of present HSYA. 15 ♀, No. 0016, parent of present HSYA. 16 ♂, No. 0025, parent of absent HSYA. 17 The bulk of absent HSYA. 18–23 segregating individuals of absent HSYA. M DL 2000 Marker

individuals in the segregating population. This established a tight linkage of the four AFLP markers to the present HSYA in safflower. Availability of the four markers lays a solid foundation for using molecular marker-assisted genetic selection in safflower breeding, making it possible to shorten the breeding cycle.

Although we identified AFLP markers in a short period of time, they are generally expensive to generate, thus limiting large-scale application in marker-assisted plant for ease of use, AFLP markers need to be converted into simple SCAR markers. This methodology involves characterization of the linked marker and design of locus-specific primers (Negi et al. 2000). The conversion of a linked marker to SCAR has been applied successfully in a number of cases involving RAPD markers (Naqvi and Chattoo 1996) and AFLP markers (Adam-blondon et al. 1994).

In our study, the polymorphic markers linked to presence of HSYA obtained after AFLP analysis were in size from 277 to 936 bp. Based on their end sequences, the primers for SCM16 produced a strong band in the presence of HSYA in parent of No. 0016 and the individuals, and it turned out to be a useful SCAR marker, namely SCM16.

To sum up, in our present study DNA fragment was successfully employed for the identification of markers associated with the HSYA content trait in Flos Carthami. AFLP is an important molecular marking technique, but to our knowledge, no report has been seen in the study of the relationship between DNA molecular marker and the content in the secondary metablite of medicinal plants. Our research has obtained the AFLP fragments and a SCAR marker linked to the content of HSYA in Flos Carthami. We hope that our findings would provide clues for evaluating the quality at the molecular level and lay a foundation for further study of directional regulation of the property of Flos Carthami.

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