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Transferability of sunflower microsatellite markers to safflower

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Abstract
Practically no molecular tools have been developed so far for safflower (*Carthamus tinctorius* L.) breeding. This contrasts with the extensive molecular research conducted in other major oilseed crops such as sunflower (*Helianthus annuus* L.). Microsatellite markers (SSRs) are increasingly becoming the molecular markers of choice for trait breeding, due to their high robustness and polymorphism, and their co-dominant score. However, advances in the use of SSRs have been difficult due to the relatively high cost and time required for their development. Several studies have shown the possibility of transferring SSR markers from one plant species to another belonging to the same genus or even to a different one. The chance of successful cross-species SSR amplification is inversely related to the evolutionary distance between the two species. The objective of this study was to analyze the transferability of SSRs from sunflower to safflower, both belonging to the Asteraceae family. One hundred and seventeen public SSR markers (ORS markers) developed for sunflower were tested in a set of six safflower breeding lines and two sunflower lines used as a positive control. Nineteen SSR primer pairs (16.2%) were found to be transferable (i.e. they showed good quality and specific SSR amplification products in safflower) between the two species. The results indicated that there is a potential for transferring SSR markers between sunflower and safflower. The identification of a set of transferable SSR markers would enable the application of the SSR technology in safflower molecular research.

Key words: Cross-species marker transferability — sunflower microsatellites — molecular markers

Introduction

Breeding programs in most oilseed crops are currently based to a large extent on the development and use of molecular tools. This is for example the case for sunflower (*Helianthus annuus* L.) (reviewed in Paniego et al., 2007), the closest relative oilseed crop to safflower. However, practically no molecular tools have been developed so far for safflower. Microsatellite markers (SSRs) are increasingly becoming the molecular markers of choice for trait breeding, due to their high robustness and polymorphism, and their co-dominant score. Advances in the use of SSRs have been difficult due to the relatively high cost and time required for their development. Alternatively, several studies have shown the feasibility of transferring SSR markers from one plant species to another belonging to the same genus or even to a different one, for example in cereals (Kuleung et al., 2004), legumes (Peakall et al., 1998), and Cruciferae (Plieske and Struss, 2001). The objective of the present research was to analyze the transferability of SSR markers from sunflower to safflower, both belonging to the Asteraceae family.

Materials and Methods

Six safflower lines were used to evaluate the transferability of sunflower SSR markers: Rancho, CL1, IASC-1, CR-6, CR-9, and CR-142. The pedigree of these lines has been reported by Velasco et al. (2005). The two sunflower lines P-21 and P-96 (Pérez-Vich et al., 2004) were used as a positive control. For DNA extraction, two fully expanded leaves were cut from plants of each line and frozen at -80ºC. The leaf tissue was lyophilized and ground to a fine powder in a laboratory mill. DNA was isolated from ground leaf tissue using a modified version of the protocol described by Rogers and Bendich (1985).

A set of 117 SSR markers developed from sunflower and selected from its public map (Tang et al., 2002) was used (Table 1). Initially, conditions used for PCR were those described for the SSR source species (sunflower). PCR reactions were performed in a final volume of 30 µl containing 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs (Invitrogen, San Diego, CA, USA), 0.3 µM of primers, 0.7 U of Taq DNA polymerase (BioTaqTM DNA Polymerase, Bioline, London, UK), and 50 ng of template DNA. Amplifications were run on a GeneAmp PCR System 9700 (Applied Bosystems, Foster City, CA, USA) under the following conditions: initial denaturation at 94ºC for 2 min, followed by 1 cycle of 94ºC for 30 sec, annealing temperature (*Ta*) recommended for the source-species amplification +10ºC for 30 sec, and 72ºC for 30 sec. The annealing temperature was decreased 1ºC per cycle during each of the 9 following cycles, at which time the products were amplified for 32 cycles at 94ºC for 30 sec, *Ta* for 30 sec, and 72ºC for 30 sec with a final extension of 20 min at 72ºC. The amplification products were resolved on 3% Metaphor (BMA, Rockland, ME) agarose gels in 1x TBE buffer with ethidium bromide incorporated in the gel.

For those SSR that failed to amplify or showed very weak amplification products, PCR conditions were optimized by adjusting reaction mixtures to varying concentrations of $MgCl_2$ (1.5, 2, 2.5 and 3 mM), primers (0.4, 0.8 and 1.2 µM), Taq DNA polymerase (1 unit), and DNA (100 ng), and by lowering the annealing temperature from 3 to 5ºC and/or using a non-touchdown PCR program.

To evaluate the transferability of sunflower SSR markers to safflower, the microsatellite products observed in safflower were classified into three classes based on the band intensity and ease of scoring: (++) strong band and easy score; (+) weak band and difficult to score; and (-) no band (Table 1). To determine the specificity of the amplification, the band size of the safflower products was compared to that of the sunflower products. The amplified fragments were classified as specific (+) if they produced amplification products with a size similar (within 100 bp) to that of sunflower (Table 1).

Results and Discussion

As described in Table 1, 33 (28.2%) of the sunflower SSR tested gave a positive amplification in safflower (scored as + and ++). From these, 19 (16.2%) showed a clear and strong PCR product (scored as ++). Amplified products were highly specific, with 31 out of 33 of them showing a band of similar size to that of the sunflower control (Table 1). These results indicated that there is a potential for transferring SSR markers between sunflower and safflower. The identification of the set of transferable SSR markers described in Table 1 represents a first step towards the application of the SSR technology to molecular studies in safflower.

The chance of successful cross-species SSR amplification is inversely related to the evolutionary distance between the two species. As a consequence, successful cross-species amplification outside a genus is much lower than that reported within a genus. For example, a range of cross-species amplification among genera within the Fabaceae family from 3% to 13% has been reported, compared to 65% within the Fabaceae genus *Glycine* (Peakall et al., 1998). Even though the transferability value (16.2%) obtained in this study is similar to those previously reported for other plant families (Peakall et al., 1998; Kuleung et al., 2004), it would not be enough to supply a critical mass of DNA markers for practical research in safflower. It might be possible to increase the success of cross-species amplification rate by using SSRs based on expressed sequence tags (ESTs), which come from transcribed regions of the genome, and are likely to be conserved across a broader taxonomic range than traditional “anonymous” SSR markers. In fact, Pashley et al. (2006) found that EST-derived SSRs were 3 times more transferable across species of the genus *Helianthus* than anonymous SSRs. These approaches, together with the development of specific safflower markers, such as anonymous SSRs (Pérez-Vich et al., unpublished results), In/Del markers based on ESTs (Chapman et al., 2007) and EST-derived SSR from the 41,011 safflower EST already available (The Compositae Genome Project, http://compgenomics.ucdavis.edu/, last accessed July 20, 2008) will contribute to increase the safflower genomic tools and will expedite safflower molecular breeding in the coming years.

Table 1. Transferability of sunflower microsatellite markers (ORS markers) to safflower.

Marker	Quality[1]	Specificity[2]	Marker	Quality[1]	Specificity[2]	Marker	Quality[1]	Specificity[2]
ORS 7	+	+	ORS 610	+	+	ORS 1030	-	-
ORS 16	++	+	ORS 613	+	+	ORS 1036	-	-
ORS 70	-	-	ORS 621	-	-	ORS 1041	-	-
ORS 90	++	+	ORS 630	-	-	ORS 1043	++	+
ORS 154	-	-	ORS 650	-	-	ORS 1065	-	-
ORS 166	-	-	ORS 656	-	-	ORS 1079	-	-
ORS 185	++	+	ORS 665	-	-	ORS 1085	-	-
ORS 202	+	+	ORS 666	-	-	ORS 1108	+	-
ORS 229	+	-	ORS 668	-	-	ORS 1114	+	+
ORS 230	-	-	ORS 674	++	+	ORS 1120	-	-
ORS 243	++	+	ORS 687	-	-	ORS 1141	-	-
ORS 297	-	-	ORS 691	-	-	ORS 1143	-	-
ORS 299	-	-	ORS 694	++	+	ORS 1146	-	-
ORS 307	-	-	ORS 695	-	-	ORS 1152	++	+
ORS 309	-	-	ORS 716	-	-	ORS 1161	+	+
ORS 311	++	+	ORS 733	-	-	ORS 1178	-	-
ORS 312	-	-	ORS 735	-	-	ORS 1179	-	-
ORS 316	-	-	ORS 750	++	+	ORS 1222	-	-
ORS 317	-	-	ORS 761	-	-	ORS 1227	-	-
ORS 328	++	+	ORS 762	++	+	ORS 1231	++	+
ORS 329	++	+	ORS 774	-	-	ORS 1245	-	-
ORS 331	-	-	ORS 778	-	-	ORS 1248	-	-
ORS 342	-	-	ORS 780	+	+	ORS 1256	-	-
ORS 366	-	-	ORS 785	-	-	ORS 1260	-	-
ORS 371	-	-	ORS 810	-	-	ORS 1265	-	-
ORS 381	+	+	ORS 826	+	+			
ORS 407	-	-	ORS 830	+	+			
ORS 420	++	+	ORS 832	-	-			
ORS 423	-	-	ORS 837	-	-			
ORS 428	-	-	ORS 844	-	-			
ORS 437	-	-	ORS 852	-	-			
ORS 442	-	-	ORS 857	-	-			
ORS 453	++	+	ORS 878	-	-			
ORS 456	-	-	ORS 885	-	-			
ORS 457	++	+	ORS 887	-	-			
ORS 483	-	-	ORS 894	-	-			
ORS 502	-	-	ORS 898	-	-			
ORS 505	-	-	ORS 899	-	-			
ORS 533	-	-	ORS 925	-	-			
ORS 534	-	-	ORS 938	-	-			
ORS 536	++	+	ORS 949	-	-			
ORS 543	-	-	ORS 963	-	-			
ORS 561	-	-	ORS 966	-	-			
ORS 565	-	-	ORS 993	-	-			
ORS 595	+	+	ORS 1013	++	+			
ORS 599	+	+	ORS 1024	-	-			

[1]Quality of the amplification in safflower: (++) strong band and easy score; (+) weak band and difficult to score; and (-) no band.

[2]Specificity of the amplification: (+) the safflower amplified product was of a similar size (within 100 bp) to that of sunflower; (-) the safflower amplified product was not of a similar size (within 100 bp) to that of sunflower.

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