

Identification of sunflower (*Helianthus annuus*, Asteraceae) hybrids using simple-sequence repeat markers

A. Iqbal, H.A. Sadaqat, A.S. Khan and M. Amjad

Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad, Pakistan

Corresponding author: A. Iqbal E-mail: ahsanuaf2003@yahoo.com

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ABSTRACT. Hybrid identification of 16 sunflower hybrids was confirmed using simple-sequence repeat methodology. Of 20 specific simple-sequence repeat primers, 18 authenticated the purity of these hybrids; the remaining two specific primer pairs gave ambiguous DNA fragments. The results indicate that simple-sequence repeat analysis for the identification of hybrids derived from the crossing of different inbred sunflower lines can improve the accuracy of selection, save time and reduce cost.

Key words: Diversity; RAPD marker; Sunflower

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INTRODUCTION

Sunflower is of great importance because its seed has high oil contents ranging from 40-50% (Skoric and Marinkovic, 1986). Only 30% of edible oil requirements of Pakistan are met through local production, and the other 70% of the country's requirements are met through importation, costing huge amounts of foreign exchange. The imported edible oils are mainly palm and soybean oil. However, the shortage of edible oil still persists. The situation of oil production in Pakistan is better than before, but due to increasing demand by the ever-increasing human population, continuous improvement in its productivity is highly desirable. For the last few years, yield improvement in sunflower varieties has not been substantial. The narrow genetic base and pure local hybrids of the germplasm used have been considered major drawbacks in the development of an ideal high-yielding local hybrid. The identification of sunflower hybrids is normally performed through morphological markers. The information about genetic diversity and hybrid identification in the available germplasm and among elite breeding material is essential in plant breeding (Mosges and Friedtu, 1994). The future breeding program depends on the availability of a pure local hybrid to increase productivity. Traditionally, the assessment of hybrid purification has been based on the differences in morphological traits. RFLP (restriction fragment length polymorphism) markers have been used for hybrid studies and genetic mapping of different crops (Hernández et al., 2000). Polymerase chain reaction (PCR)-based DNA marker techniques seem to provide the means for generating useful information for hybrid purification, genetic relatedness and diversity. PCR-based simple-sequence repeat (SSR) markers are co-dominant markers and extensively used in hybrid identification (Chalmers et al., 2001), and the identification of markers is linked to different traits (Bai et al., 2003). SSR methodology has been used for pure hybrid analyses in several crops (Li and Nelson et al., 2001). SSR analysis was carried out in 16 genotypes of sunflower for hybrid identification and to determine purity among them, and to compare these 16 genotypes with their 8 parents. The use of these markers frequently results in the exclusion of hybrid seedling from segregating populations, which is considered highly undesirable in breeding program.

MATERIAL AND METHODS

This study included 8 parents of sunflower and their 16 hybrids. The sunflower parents were CM-612, HA-27, B-SIN-82, HA-314, RL-54, RL-51, R-SIN-82, and RL-46, and their 16 hybrids were designated hybrid-1, hybrid-2hybrid-16. The genotypes were grown in plastic containers. The temperature during germination was 35° C in the growth chamber and light was supplied for 16 h. Leaf tissues, 0.2-0.3 g, were obtained from the 6-day-old leaves of the sunflower genotypes. The weighed leaves were then immediately transferred to plastic zipper bags containing 1.5 mL CTAB (Khan et al., 2004). Finally, the concentration of DNA was measured at 260 nm in a spectrophotometer (CECIL CE 2021 2000 Series). The quality of DNA was checked by running 5 μ L DNA on a 1.2% agarose gel prepared in 0.5X TBE buffer. The DNA samples giving a smear on the gel were rejected.

PCR amplification

DNA concentration in the working solution of approximately 15 ng/µL in d3H₂O was

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confirmed by spectrophotometry. For SSR analysis (Williams et al., 1990), the concentrations of genomic DNA, 10X PCR buffer with $(NH_4)2SO_4$, MgCl₂, dNTPs (dATP, dCTP, dGTP, dTTP), 10-mer random primer and Taq DNA polymerase (Fermentas) were optimized. DNA amplification reactions were performed in a thermal cycler (Eppendorf AG, Hunberg). The PCR profile was as follows: one cycle of 94°C for 5 min, 35 cycles of 94°C for 1 min, 36°C for 1 min and 72°C for 2 min, and a final extension for 10 min at 72°C. The SSR fragments were separated by electrophoresis on 1.2% agarose gels with ethidium bromide (10 ng/100 mL of agarose solution in TBE). A 100-bp ladder was also loaded along with the DNA to check the size of the DNA fragments of the parents and the hybrid (Figure 1). All 16 hybrids showed an exact relationship with their parents. Only two SSR primers showed ambiguous bands, which may be due to the undesired annealing temperature or due to the non-optimal concentration of MgCl₂.



Figure 1. Hybrid identification of sunflower using SSR primer ORS 324. M = molecular marker; P_1 and P_2 =

RESULTS AND DISCUSSION

DNA of 8 sunflower parents and their 16 hybrids was amplified with 20 different specific primer pairs (Table 1). The number and size of the DNA fragments were strictly dependent on the sequence of the primer. Reactions were repeated two to three times to check the consistency of the amplified products, and only easily resolved and bright DNA bands were counted. All F1 genotypes showed an authentic confirmation with their parents.

These results suggest that SSR markers provide information for the identification of sunflower hybrid genotypes (Lawson et al., 1994). The reproducibility of the SSR technique can be influenced by various factors, such as sequence of a primer, template quality and quantity, the type of thermocycler and polymerase concentration (Hernández et al., 1999). However, the use of a standardized SSR protocol can ensure a reproducible SSR pattern.

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Identification of sunflower hybrids using SSR markers

ORS 296 CCTTGCACTTAGCCCA GCATTCACAACAAACATCATCA ORS 290 TCTTTACTTCCACGGTGCACTA GCATTCACAACAAACATCATCA ORS 287 CGGATTCACTGCTTTCCAAT GCATTGCCACACAGAGAAA ORS 300 GAATGCGGAGACAAAGGCT ATAAGTGTGGCGCTGGAAAC ORS 300 CATTGGATGGAGCACTTT GATGAGGGAAATCGTG ORS 310 CGTGACCTGTGAAACACCAA CGATAACCGTGTGAAATGGGGCAACTTG ORS 310 AATTCCCACGCAAACTTCAA GGGTAAAATGGGGCAACCTAT ORS 311 TCCCGAAATAGGGGATTGA GATTGGGTGTGGGGCACCTAT ORS 316 TGGCGTCTTCATAGCCAAAGAAC GGGTAGGTTGTGGGTGAGCTTGTGGGA ORS 318 TCCATGAGTTGCGGCGTATGC CCGCCATATTGAAACTCCATAC ORS 321 TGTCGAAAGATTGCGGAAC GGGAAGGTGAAACCCTAAC ORS 322 TGCACCACTTGGAACTTGCAC GGGAAGGTAAACCGCTAAC ORS 322 TGCACCACTTGGAACTTGCAC GCGGAAGTAAGAGGGT ORS 332 CGGGAAACTAGGATCAGAGG GCCGGGAGGTTAAGAGGGTT ORS 333 CGGTTAAGAGGTCATGGTCGCAAC GGGAAGGTGAAACCACTAAC ORS 333 CGGTAACGCACACTTGCACCACTAC TGACACCACCTGGAACTGGTGGTCA ORS 333 CGGTAAGCAGCACACA AAACCGGCCTCTTATTGGTTACACCACACACGCTACCCCTAACC ORS 339 CCCTCTTCC	$\begin{array}{c ccccc} ORS 296 & CCTTGCACTTAGCCCA & GCATTCACAACAAACATCATCA \\ ORS 290 & TCTTTACTTCCACGGTGCACTA & GCATAGTGCCCAACAAAACATCATCA \\ ORS 300 & GAATGCGGAAGAAAGGCT & ATAAGTTGCGCCGTCAGAAGA \\ ORS 301 & CCTGACCTGTGAAACACCAA & GCATAACCGTGAAACATCCTG \\ ORS 309 & CATTGGATGGAGCCACATT & GATGAGAGTGGGGAATTTGTG \\ ORS 310 & AATTCCCACGCCAAACTTCAA & GGTAAATGGGGCAACCTAT \\ ORS 311 & TCCCGAATTAGCCAAAGAAC & GGTAAATGGGGCAACCTAT \\ ORS 315 & GCCGTGAATAATGGGATTGA & GATTGGGTCAGCTTGTGTGGA \\ ORS 316 & TGCCGTGAATAATGGGATCAG & GAATTGGGTCAACCTTGAACTGCAG \\ ORS 318 & TCCATGAGTGTGGTGTGTGC & CCGCCATATTGAAACCGCTAAC \\ ORS 319 & TCATCAATCCAAGCACCAAA & TTGGGCCGTAAACCCTTAAC \\ ORS 322 & TGCACCACTTGGAAC & GCATTCATCCATAGCACAAGGAGTT \\ ORS 323 & CGGGAAACTAGGATCAGAGG & GCCGTGGAAACCCTTAAC \\ ORS 323 & CGGGTAAACGGCTCATTGAC & GCATTCATCCATAGGCATCCAGAGG \\ ORS 333 & CGGTTAAGAGGTCAGAGG & GCCGGGAGATTAGGAGGAGTT \\ ORS 332 & CGGGTAAACGCGCGCATTTGAC & AAACCGGCCCCTTATTTGGGT \\ ORS 333 & CGGTTAAGATGGTCCAGTGGG & ATATTAAGTTTTGGTTTTAGCCAA \\ ORS 337 & TTGGTCATCATCCTGTGTG & ATATCGCCCCCAACTCATCCCAATATGC \\ \\ ORS 339 & CCCCTTCTCCTCTCCTCTCTCTCACAA & AAACCGGCCCCCAATTTGAACCCCAATATGC \\ \\ M & P_1 & H & P_2 & P_1 & H & P_2 & P_1 & H & P_2 \\ P_1 & H & P_2 & P_1 & H & P_2 & P_1 & H & P_2 \\ \end{array}$	ORS 296 CCTTGCACTTAGCCCA GCATTCACAACAAACATCATCA ORS 287 CGGATTCACTGCTTTCCCAAT GCATTCACAACAACAACATCATCA ORS 300 GAATGCGGAGACAAAGGCT ATAACTGTGGCCGTGGAAGA ORS 301 CGTGACCTGTGAAACACCAA GATAACGGGGGGAGACAATGGTG ORS 300 CATTGGATGGAGCCACTTT GATGAAGATGGGGGAATCATCAG ORS 310 AATTCCCCAGCAAACTCCAA GGTATAGCGGGGGGGACCACTTT ORS 311 TCCCCGAATTAGCCAAAGAAC GGTGTGGGGTGTGCAGCATT ORS 311 TCCCCGAATTAGCCAAAGAAC GGTGTGGGGTGTTCAGCACCACTT ORS 311 TCCCCGAGTTGGCCGATAGC GGGGGGGGAACCTTTGGA ORS 318 TCCATCAATCCAAGCACCAAA TGGGGCAACCTTAAC ORS 319 TCATCAATCCAAGCACCAAA TGGGGCAAACCTTAAC ORS 322 TGCACCACTTGGAACCACGAAC GGAATTCATCAATCCAAGGAGT ORS 323 CGGGAAACTAGGATCAGAGAG GCCGGGGAGATTAGAGGGGTT ORS 324 CACTTCAATCCCAATCTCCATCTCTCTCATCAA ATGATGCTCCCCAACGGTT ORS 333 CGGTTAAGATGGTCAGCTAGG GGTGTGTGGTGTTCATTGGC ORS 333 CGGTTAAGATGGTTCAGTTGG ATATAAGTTTGGTGTGTGTGTTTATTCGTC ORS 333 CCGTTAAGATGGTCACTTACTT AAATCCGCACACCCAATTGG ORS 333 CCCTTCTCTCTCCTTA	ORS 296 CCTTGCACTTAGCCCA GCATTCACACAACAACATCATCA ORS 297 CGGATCCACTGCCTTCCCAT GCATGCCCCCCCAGGAGAA ORS 300 GAATGCGGAGACAAAGCCCAA GCATGCTGCCCCCCCAGGAGAA ORS 300 CATTGGATGGAGCACCTTT GCATGCTGCCGTGGGAAACCCGA ORS 300 CATTGGATGGAGCACCTTT GATGCTGGCGGGGACAAACGCCAA ORS 310 ATTCCCACGCAAACTCAA GGTGTGGGTGTGTGCAGCCACTTG ORS 311 TCCCGAATAAGGCAACAG GGTGTGGGTGTGCGTGTGCAGCTGGGGAACCAGG ORS 315 GCCGTGAATAATGGGATGA GGTGTGGGTGTGCGTGCGCACCTTA ORS 316 TGCCGGGATAATGGGATCGA GGGAAGCTTGGGAAACCCCTAAC ORS 312 TGTGAAAGTGGGACACCCAAA GGAATGGGCAACCCTAACC ORS 322 TGCACCAGTGGAACTAGGAG GGGAAGGTAAGAGG ORS 323 CGGGAAACTAGGAGCAACCAGAA GCCGGAGGATTAGGAGGAACCAGGAACCAGGAACCAGGAACCAGGAACCAGAGTGAGAGG ORS 333 CGGTAAACTGGTTCAGTGG ATACCGCCCTCTAATTGGC ORS 333 CGGTAAACTGGTTCCATGGGTCAACTGG GCGGAGGTAGAAGAGAGAACTAGGAAC ORS 333 CGGTAACTGGTTCCATGCATGGG GCGGAGGTTAGAGAGGG ORS 333 CGGTAACTGGTTCCATGCTGG ATACCGCCCTCATATGGAGACACCAAA ORS 333 CGCTTCATCATCCTTGGTGG ATATAATGGGGTAACTAGGAG	No.	1	Primer n	ame	 Forward	sequence	9			Reverse	sequence		
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ORS 315 GCCOTIOAATAAGOGGATICAA GATTOGOTICAGCTTIGGTGTGTGT ORS 316 TGGCGTCTTCATAGCATCAG GAGATTTGAAGCTTCGTGTTGC ORS 318 TCCATCAATCCAAGCACCAAA TTGGGCCGTAAACCCTTAAC ORS 321 TGTCGAAGAGTTGTCGGAAC GGGAAAGTGGAACCCTAACC ORS 322 TGCACCACTTGGAACTTGGAC GGGAAAGTGAACCCCTAAGC ORS 323 CGGGAAACTAGGATCAGAGG GCCGGAGGATTAGAGGGAGTT ORS 324 CACTTCTACTCCATCTTCTTCATCAA ATGATGCTCCGCAACAGTTT ORS 332 GACCAGCCGCCATATTTCAA AAACCGGCCTCTTATTTGGT ORS 333 CGGTTAAGATGGTTCAGTTGG ATATTAAGTTTTGGTTTTAGCCAA ORS 337 TTGGTTCATTCCTCCTTGCTT GGGTTGGTGGTTAATCGTC ORS 339 CCCTCTTCCTCTCCCTTACTTT AAATCCGCACTCCAATATGC	M P ₁ H P ₂ P ₁ H P ₂ M P ₁ H P ₂	M P ₁ H P ₂	M P ₁ H P ₂ P ₁ H P ₂ M P ₁ H P ₂			ORS 3	11 15	CCCCT			JAAC TCA		GGIGI	CCTCA	TIGCAG	CTCA
ORS 316 HOCOTIC HEARGENERGE GAOATH TOCOTIC HEARGENERGE ORS 318 TCCATGAAGCATCAC GCGCATATATGCATCTGCATC ORS 319 TCATCAATCCAAGCACCAAA TTGGGCCGTAAACCCTTAAC ORS 321 TGTCGAAGAGTTGTCGGAAC GGGAAGGTGAAACCCTAACC ORS 322 TGCACCACTTGGAACTTGAC GCGGAAGACTACCAAGAG ORS 323 CGGGAAACTAGGATCAGAGG GCCCGGAGGATTAGAGGAGTT ORS 324 CACTTCTACTCCATCCTTCTTCTACACAA ATGATGCTCCCGCAACAGTTT ORS 332 GACCAGCCGCCATATTTCAA ATGATGCTCCGCAACACGTTT ORS 333 CGGTTAAGATGGTTCAGTTGG ATATTAAGTTTTGGTTATGCCAAGTTG ORS 337 TTGGTTCATTCATCCTTGGTC GGGTTGGTGGTGGTGATTAGCC ORS 339 CCCCTCTTCCTCTCCCTTACTTT AAATCCGCACTCCAATATGC M P_1 H P_2 P_1 H P_2 M P_1 H P_2 P_1 H P_2 P_1 H P_2	M P ₁ H P ₂	M P ₁ H P ₂ P ₁ H P ₂ M P ₁ H P ₂	M P ₁ H P ₂ P ₁ H P ₂ M P ₁ H P ₂			ORS 3	15	TCCCC	JAAIAA		TUA		CACAT	TTCAC	TTCCT	CTTCC
$\begin{array}{c} \text{ORS 319} & \text{TCATCAATCCAAGCCAAAA} \\ \text{ORS 321} & \text{TGTCGAAGCATGCAGCAAAA} \\ \text{ORS 322} & \text{TGCACCACTTGGAACTTGAC} \\ \text{ORS 322} & \text{TGCACCACTTGGAACTTGAC} \\ \text{ORS 323} & \text{CGGGAAACTAGGATCAGAGG} \\ \text{ORS 324} & \text{CACTTCTACTCCATCTTTTCAAACGCCGCAACAA} \\ \text{ORS 332} & \text{CGGTTAAGATGGTTCAGTTGG} \\ \text{ORS 333} & \text{CGGTTAAGATGGTTCAGTTGG} \\ \text{ORS 337} & \text{TTGGTTCATTCATCCTTGGTC} \\ \text{ORS 339} & \text{CCCCTCTTCCTCTCTCTTCACTTA} \\ \end{array}$	M P ₁ H P ₂ P ₁ H P ₂ M P ₁ H P ₂	M P ₁ H P ₂	M P ₁ H P ₂			ORS 3	10	TCCAT	AGTTG	GTCGTN	LAG		CCGC	TATTGA	AACTG	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	ORS 321 TGTCGAAGAGTTGTCCGGAAC GGGAAGGTGAAACCCTAACC ORS 322 TGCACCACTTGGAACTAGAG GGGAAGGTGAAACCCTAACC ORS 323 CGGGAAACTAGGATCAGAGG GCCGGGAGATTAGAGGGAGTT ORS 324 CACTTCTACTCCATCTTCTTCATCAA AGCCGGCCGCATATTTGGG ORS 333 CGGTTAAGATGGTTCAGTTGG ATATTAAGTTTTGGTTTTAGCCAC ORS 333 CGGTTAAGATGGTTCAGTTGG ATATTAAGTTTTGGTTATTGGT ORS 337 TTGGTTCATTCATCCTTGGTC GGGTTGGTGGTGGTGAAACCCCAATATGC ORS 339 CCCCTCTTCCTCTCCCTTACTTT AAACCGGCACATTGCGC M P1 H P2 P1 H P2 P1 H P2	M P ₁ H P ₂	M P ₁ H P ₂ P ₁ H P ₂ M P ₁ H P ₂			ORS 3	10	TCATCA					TTGGG	CCGTA	AACTO	
ORS 322 TGCACCACTTGGAACTTGAA GGATTCATCATCATGAAGAG ORS 323 CGGGAAACTAGGATCAGAGG GCATTCATCCCATAGTCATCAGAGAGG ORS 324 CACTTCTATCCATCATCATCAA ATGATGCTCCGCAACAGTTT ORS 332 GACCAGCCGCATATTTCAA ATGATGCTCCGCAACAGTTT ORS 333 CGGTAAAGAGGGTTCAGTTGG ATATAAGTTTTGGTTATCAGCAA ORS 337 TTGGTTCATCATCCTTGGTC GGGTTGGTGGTTAATCGTC ORS 339 CCCTCTTCCTCTCCTTACTTT AAACCGGCATCCAATATGC M P1 H P2 P1 H P2 P1 H P2	M P ₁ H P ₂	M P ₁ H P ₂	M P ₁ H P ₂			ORS 3	21	TGTCG	AGAGI	TGTCG	GAAC		GGGA	AGGTGA		
ORS 323 CGGGAAACTAGGATCAGAGG GCCGGAGGATTAGAGGAGTT ORS 324 CACTTCTACTCCATCTTCTTCATCAA ATGATGCTCCGCAACAGTTT ORS 332 GACCAGCCGCATATTTCAA AACCGGCCTCTTATTTGGT ORS 333 CGGTTAAGATGGTTCAGTTGG GGGTTGGTGGTGGTGATTGGCTC ORS 339 CCCTCTTCCTCTCCCTTACTTT AAATCCGCACTCCAATATGC M P1 H P2 P1 H P2 P1 H P2	ORS 323 CGGGAAACTAGGATCAGGG GCCGGAGGATTAGAGGAGATTA ORS 324 CACTTCTACTCCATCTTCTTCATCAA GCCGGGAGGATTAGAGGAGTTA ORS 332 GACCAGCCGCATATTTCAA AGACCGGCCTCTTATTTGGT ORS 333 CGGTTAAGAGGGTTCAGTTGG AACCCGGCCTCTTATTTGGT ORS 337 TTGGTTCATTCATCCTTGGTC GGGTTGGTGGTTAATTCGC ORS 339 CCCTCTTCCTCTCCCTTACTTT AAATCCGCACTCCAATATGC M P1 H P2 P1	MP1 HP2 P1 HP2 P1 HP2 P1 HP2 P1 HP2 P1 HP2 P1 HP2	M P ₁ H P ₂			ORS 3	21	TGCAC	CACTTG	GAACTI	GAC		GCATT	CATCCA	TAGTCA	TCAAGA
$\begin{array}{ccccccc} & \text{ORS 324} & \text{CACTTCTACTCCATCTTCTTCATCAA} \\ & \text{ORS 332} & \text{GACCAGCCGCATATTTCAA} \\ & \text{ORS 333} & \text{CGGTTAAGATGGTTCAGTTGG} \\ & \text{ORS 337} & \text{TTGGTTCATTCATCCTTGGTC} \\ & \text{ORS 339} & \text{CCCTCTTCCTCCCCTTACTTT} & \text{AACCGGCCTCTTATTGGTTAAGCCAGGGTTGGTGGTGGTGGTGGTGAATATCGTC} \\ & \text{M} & \text{P}_1 & \text{H} & \text{P}_2 \\ \hline & \text{M} & \text{P}_1 & \text{H} & \text{P}_2 & \text{P}_1 & \text{H} & \text{P}_2 & \text{P}_1 & \text{H} & \text{P}_2 \\ \hline & \text{ORS 339} & \text{CCCTCTTCCTCCCTTACTTT} & \text{AATCCGCACTCCAATATGCC} \\ \end{array}$	ORS 324 CACTTCTACTCCATCTTCTTCATCAA ATGATGCTCCGCAACAGTTT ORS 332 GACCAGCCGCATATTTCAA ATGATGCTCCGCCACAGTTT ORS 333 CGGTTAAGATGGTTCAGTTGG ATGATGCTCCGCCACAGTTT ORS 337 TTGGTTCATTCATCCTTGGTC AACCGGCCTCTTATTTGGT ORS 339 CCCTCTTCCTCTCCCTTACTTT AAATCCGCACTCCAATATGC M P1 H P2 P1 H P2 P1 H P2	ORS 324 CACTTCTACTCCATCTTCTTCATCAA ATGATGCTCCGCAACAGTTT ORS 332 GACCAGCCGCATATTTCAA ATGATGCTCCGCCACAGTTT ORS 333 CGGTTAAGATGGTTCAGTTGG AACCGGCCTCTTATTTGGT ORS 337 TTGGTTCATTCATCCTTGGTC AAATCCGCACTCCAATATGCC ORS 339 CCCTCTTCCTCTCCCTTACTTT AAATCCGCACTCCAATATGCC M P1 H P2 P1 H P2 P1 H P2	ORS 324 CACTTCTACTCCATCTTCTTCATCAA ATGATGCTCCGCAACAGTTT ORS 332 CGGTTAAGATGGTTCAGTTGG ATGATGCTCCGCCAACAGTTT ORS 333 TTGGTTCATTCATCCTTGGTC CGGTTAAGATGGTTCAGTTGGC ORS 339 CCCTCTTCCTCTCCTCCCTTACTTT AATCCGCACTCCAATAGCC M P1 H P2 P1 P3 P3			ORS 3	23	CGGGA	AACTA	GATCA	GAGG		GCCG	GAGGAT	TAGAGO	GAGTT
ORS 332 GACCAGCCGCATATTTCAA AAACCGGCCTCTTATTTGGT ORS 333 CGGTTAAGATGGTTCAGTTGG ATATTAAGTTTTGGTTTTAGCCAT ORS 337 TTGGTTCATTCATCCTTGGTC GGGTTGGTGGTGGTGGTGGTGATTCGTC ORS 339 CCCTCTTCCTCTCCCTTACTTT AAACCGGCCTCTTATTTGGT M P1 H P2 P1 H P2 P1 H P2	ORS 332 GACCAGCCGCATATTTCAA AAACCGGCCTCTTATTTGGT ORS 333 CGGTTAAGATGGTTCAGTGG ATATTAAGTTTTGGTTATAGCCAG ORS 337 TTGGTTCATTCATCCTTGGTC AAACCGGCCTCTTATTTGGTTATAGCCAG ORS 339 CCCTCTTCCTCTCCCTTACTTT AAACCGGCCTCTAATTCGTC M P1 H P2 P1 H P2 P1 H P2 M P1 H P2 P1 H P2 P1 H P2	ORS 332 GACCAGCCGCATATTTCAA AAACCGGCCTCTTATTTGGT ORS 333 CGGTTAAGATGGTTCAGTTGG ATATTAAGTTTTGGTTATAGCCAT ORS 339 CCCTCTTCCTCTCCCTTACTTT AAACCGGCCTCTTATTTGGT M P1 H P2 P2	ORS 332 GACCAGCCGCATATTTCAA AAACCGGCCTCTTATTTGGT ORS 333 CGGTTAAGATGGTTCATCCTTGGTC ATATTAAGTTTTGGTTATTGCCAG ORS 339 CCCTCTTCCTTCCCTTACTTT AAACCGGCACTCCAATATGC M P1 H P2 P2 P2 P3 P3 P3 P3 P3 P3 P3 P3 P3 P4			ORS 3	24	CACTT	CTACTC	CATCTT	CTTCATC	CAA	ATGAT	GCTCCG	CAACA	GTTT
ORS 333 ORS 337 ORS 339 CGGTTAAGATGGTTCATCATTGG CCCTCTTCCTCTCCCTTGGTC AAATCCGCACTCCAATATGC M P ₁ H P ₂ P ₁ H P ₂ P ₁ H P ₂ P ₁ H P ₂ M P ₁ H P ₂	ORS 333 ORS 337 ORS 337 ORS 339 CCGGTTAAGATGGTTCATCATCGTGG CCCTCTTCCTCCCCTTACTTT MP1 H P2 P1 H P2 P1 H P2 P1 H P2 M P1 H P2 P1 H P2 P1 H P2 P1 H P2	ORS 333 ORS 337 ORS 339 CGGTTAAGATGGTTCAGTGG TTGGTTCATCCATCCTTGGTC CCCTCTTCCTCTCCCTTACTTT ATATTAAGTTTTGGTTTTAGCCAG GGGTTGGTGGTGGTGATTCGTC AAATCCGCACTCCAATATGC M P1 H P2 P1 H P2 P1 H P2	ORS 333 ORS 337 ORS 339 CGGTTAAGATGGTTCAGTGG TIGGTCATICATCCTIGGTC CCCTCTTCCTCTCCCTTACTTT ATATTAAGTTTTGGTTTTAGCCAC GGGTTGGTGGTGGTGGTGATATTCGTC AAATCCGCACTCCAATATGC M P1 H P2 P1 H P2 P1 H P2			ORS 3	32	GACCA	GCCGCA	TATTTC	AA		AAACO	GGCCT	CTTATT	IGGT
ORS 337 ORS 339 TTGGTTCATTCATCCTTGGTC GGGTTGGTGGTGGTTAATTCGTC AAATCCGCACTCCAATATGC M P ₁ H P ₂ P ₁ H P ₂ P ₁ H P ₂ P ₁ H P ₂ M P ₁ H P ₂	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	ORS 337 ORS 339 TTGGTTCATTCATCCTTGGTC CCCTCTTCCTCTCCCTTACTTT GGGTTGGTGGTTAATTCGTC AAATCCGCACTCCAATATGC M P1 H P2 P1 H P2 P1 H P2 M P1 H P2 P1 H P2 P1 H P2 P1 H P2			ORS 3	33	CGGTT	AGATG	GTTCAG	TTGG		ATATT	AGTTT	TGGTTT	TAGCCA
ORS 339 CCCTCTTCCTCTCCCTTACTTT AAATCCGCACTCCAATATGC $M P_1 H P_2 P_1 H P_2 P_1 H P_2 P_1 H P_2$	ORS 339 CCCTCTTCCTCTCCCTTACTTT AAATCCGCACTCCAATATGC $M P_1 H P_2 P_1 H P_2 P_1 H P_2 P_1 H P_2$	$M P_1 H P_2 P_1 H P_2 P_1 H P_2 P_1 H P_2 P_1 H P_2$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$			ORS 3	37	TTGGT	CATTC/	ATCCTTC	GGTC		GGGTT	GGTGG	TTAATT	CGTC
M P ₁ H P ₂	M P ₁ H P ₂	M P ₁ H P ₂	M P ₁ H P ₂		ORS 339			CCCTCTTCCTCTCCCCTTACTTT						CGCAC	ICCAAT/	ATGC
				10.00												
																55

Figure 2. Hybrid identification of sunflower using SSR primer ORS 316. M = molecular markes; P_1 and P_2 = ; H = _____.

Different concentrations of $MgCl_2$, Taq DNA polymerase and concentration of template DNA were optimized for PCR conditions. DNA concentrations of 5, 10, 15, 20, and 25 ng/25 µL in each reaction were studied. A concentration of 10 ng/25 µL was found to produce the most consistent and reproducible banding patterns. Murray et al. (1980) used the SSR technique to evaluate some mutants and found that a 3 mM concentration was optimal for

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better amplification. In this study, 3 mM $MgCl_2$ was found to be optimal for consistent results. More than 3 mM $MgCl_2$ produced nonspecific amplification. Similarly, one unit concentration of Taq DNA polymerase was found to be optimal for better amplification of genomic DNA. Other reaction conditions were also kept constant, and the results were found to be consistent and reproducible. All amplified bands were identical in each repetition. The SSR technique used here was found to be quite effective in determining the genetic authenticity among sunflower parents and hybrids. By knowing about the authenticity of sunflower hybrid lines, a plant breeder can use this for further breeding programs.

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