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Molecular mapping of the *Rf1* gene restoring pollen fertility in PET1-based F_1 hybrids in sunflower (*Helianthus annuus* L.)

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Abstract Up to now a single cytoplasmic male sterility (CMS) source, PET1, is used worldwide for hybrid breeding in sunflower. Introgression of the restorer gene *Rf1*, responsible for fertility restoration, into new breeding material requires tightly linked markers to perform an efficient marker-assisted selection. A survey of 520 decamer primers by bulked segregant analyses identified five RAPD markers linked to the restorer gene *Rf1*. In a F_2 population of 183 individuals one of the RAPD markers, OPK13_454, mapped 0.8 cM from *Rf1*, followed by OPY10_740 with 2 cM. Bulked segregant analyses using 48 AFLP primer combinations identified 17 polymorphisms, which could be mapped in the same linkage group as *Rf1*. E33M61_136, and E41M48_113 were mapped 0.3 cM and 1.6 cM from the gene, respectively. Conversion of E41M48_113 into a sequence-specific marker resulted in a monomorphic pattern. However, two of the RAPD markers, OPK13_454 and OPY10_740, were successfully converted into SCAR markers, HRG01 and HRG02, which are now available for marker-assisted selection. To investigate the utility of these SCAR markers in other cross-combinations they were tested in a set of 20 lines. Comparison of the patterns of 11 restorer and nine maintainer lines of PET1 demonstrated that the markers OPK13_454/HRG01 and HRG02 were absent in all maintainer lines but present in all restorer lines, apart from the high oleic line RHA348 and the dwarf line Gio55. In addition, restorer lines developed from the interspecific hybrids *Helianthus annuus* × *Helianthus mollis* and *H. annuus* × *Helianthus rigidus* gave the same characteristic amplification products.

Keywords CMS · Fertility restoration · *Helianthus annuus* · SCAR markers · Sunflower

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Introduction

Fertility restoration by dominant nuclear genes is essential for hybrid breeding based on CMS to obtain high yields of seeds. In sunflower, only a single sterile cytoplasm, PET1, is used in commercial hybrid breeding. This male sterility type was created by an interspecific cross, *Helianthus petiolaris* × *Helianthus annuus* (Leclercq 1969). The molecular mechanism leading to male sterility has been well characterised for this CMS type, as the mitochondrial DNA of the male-sterile and -fertile lines differs only in about 17 kb (Siculella and Palmer 1988; Köhler et al. 1991). A new open reading frame, *orfH522* (Köhler et al. 1991; Laver et al. 1991), and the corresponding gene product the 16-kDa protein (Horn et al. 1991; Laver et al. 1991), were associated with the male-sterile phenotype. The 16-kDa protein, which is also expressed in nine other CMS sources in sunflower, is membrane-bound (Horn et al. 1996). The PET1-cytoplasm causes premature programmed cell death (PCD) of the tapetal cells, which then tends to extend to other anther tissues (Balk and Leaver 2001). Fertility restoration involves the anther-specific reduction of the co-transcript of *orfH522* and the *atpA* gene, as well as of the CMS-associated protein (Monéger et al. 1994). The tissue-specific increase in the level of polyadenylated *atpA-orfH522* transcripts was correlated with the tissue-specific instability of *atpA-orfH522* mRNAs in the androeceum of the fertility restored hybrid plants (Gagliardi and Leaver et al. 1999). Polyadenylation induces the degradation of polyadenylated RNA substrates by the activity of RNase 2, which is present in the sunflower mitochondria. The specific control of polyadenylation of *atpA-orfH522* mRNAs in florets of fertility restored hybrids might be associated with the action of one of the two restorer genes in sunflower (Gagliardi and Leaver et al. 1999).

A number of restorer lines with known pedigree (Korell et al. 1992) are available for PET1. One to four dominant restorer genes depending on the cross combination have been described (Serieys 1996). However, in

most cultivated lines two dominant nuclear genes *Rf1* and *Rf2* are responsible for fertility restoration (Leclercq 1984). *Rf2* was described to be present in nearly all inbred lines, including maintainers of CMS, and only the second gene is introduced by the restorer lines to produce fertile sunflower hybrids. This *Rf1* gene was mapped on linkage group 6 in the RFLP map and the consensus map (Gentzbittel et al. 1995, 1999) together with the *Pl5* locus conferring resistance to downy mildew (Bert et al. 2001).

The improvement of hybrid performance and the introduction of resistances to different fungal pathogens, like e.g. *Sclerotinia sclerotiorum*, from wild species into the cultivated sunflower requires a continuous development of new lines. In these breeding programmes cytoplasmic male-sterile lines are often used to guarantee cross-pollination. However, the development of new lines requires fertility restored plants that can only be identified and selected after flowering, which is time- and cost-intensive. Therefore, the availability of closely linked PCR-based markers for the fertility restoration gene(s) would be a considerable advantage for sunflower breeding. Molecular characterisation by RFLP and AFLP has been performed for sunflower lines of known restorer and maintainer pools (Berry et al. 1994; Gentzbittel et al. 1994; Hongtrakul 1997) to validate the pools and to be able to predict hybrid performance on the basis of genetic similarities (Cheres et al. 2000), but not with special emphasis on identifying markers linked to the fertility restoration gene(s).

In this study, we were interested in developing PCR-based markers for the restorer gene *Rf1* of sunflower hybrids based on the PET1 cytoplasm. We constructed a linkage map around the *Rf1* gene using the AFLP and RAPD techniques and identified markers tightly linked to the gene. These PCR-based markers were converted into easy to handle SCAR markers that are now available as useful tools for marker-assisted backcross programmes in sunflower.

Materials and methods

Plant materials

F_2, F_3 and F_2BC_1 populations (backcrossed on male-sterile HA89 carrying PET1 cytoplasm) were derived from the cross RHA325 × HA342. RHA325 is a restorer line carrying the PET1 cytoplasm; HA342 is a high-oleic maintainer line (Table 1). F_2, F_3 and F_2BC_1 populations were grown in the field of Groß-Gerau near Frankfurt/Main and evaluated for male fertility/sterility. Leaf material from F_2 individuals for DNA analyses was immediately frozen in liquid nitrogen and stored at –20 °C. The F_2 population used to map the restorer gene *Rf1* consisted of 183 individuals.

In addition to the restorer line RHA325, seven open American lines, RHA265, RHA348, CM587, CM592, CM596, CM610 and Gio55, and three inbred lines developed from interspecific hybrids (Kräuter et al. 1991) were included in the investigations (Table 1). On the maintainer side, DNA analyses were performed with the lines HA342, HA89, HA291, HA323, HA350, HA850, CM594, CM603 and CM611.

Isolation of genomic DNA

Genomic DNA was isolated according to Doyle and Doyle (1990). In liquid nitrogen ground powder of 2.5 g of leaf material was incubated with 15 ml of extraction buffer (100 mM of Tris/HCl pH 8.0, 1.4 M of NaCl, 20 mM of EDTA, 2% CTAB, 1% $Na_2S_2O_3$) at 65 °C for 30 min. After chloroform extraction the aqueous phase was obtained by centrifugation. The procedure was repeated and finally the DNA was precipitated in the aqueous phase by adding 1 ml of ammonium acetate (10 M) and 1 ml of sodium acetate (3 M, pH 5.5) in addition to two-thirds volume of 2-propanol at 4 °C. High-molecular-weight DNA was transferred by a glass hook to a new tube and washed once with wash alcohol (70% ethanol, 10 mM of ammonium acetate). DNA that was shortly dried was then solubilized in 1 ml of TE (10 mM of Tris/HCl pH = 8.0, 1 mM of EDTA).

Table 1 Major traits and pedigrees of the investigated maintainer and restorer lines (Korell et al. 1992). M: maintainer, R: restorer, n: conventional fatty acid pattern, ho: high oleic

Line	Oil type	Fatty acids	CMS	Origin	Pedigree
HA342	Oil	ho	M	USA	BC_1F_4 fom HA89*2/Pervenets
HA89	Oil	n	M	USA	from CM303 (VNIIMK 8931)
HA291	Oil	n	M	USA/France	INRA 6501
HA323	Oil	n	M	USA	F_8 from Sundak
HA350	Non-oil	ho	M	USA	BC_1F_4 from HA292*2/Pervenet
HA850	Oil	n	M	USA/UdSSR	S_{12} of high oleic populations
CM594	Oil	n	M	Canada	S_{10} from Armavirec
CM603	Oil	n	M	Canada	F_6 from CM565/CM338
CM611	Oil	n	M	Canada	HA301
RHA325	Non-oil	n	R	USA	F_7 from R818-3
RHA265	Oil	n	R	USA	selfed F_1 from Peredovik/CM953
RHA348	Oil	ho	R	USA	BC_1F_4 from RHA274*2/Pervenets
CM587	Oil	n	R	Canada	CM469*2/USDA-R-Line Pool
CM592	Oil	n	R	Canada	BC_3F_5 from RHA274/CM497*4
CM596	Oil	n	R	Canada	F_4 from AC1611/CM469/RHA297/CM497
CM610	Oil	n	R	Canada	F_5 from AC1611/CM469/RHA297/CM497
Gio55	Oil	n	R	Italy/Romania	207A(USDA)/C22 (Romanian population)
sf1578	Oil	n	R	Germany	S_6 of HA89(cms) × RIG1848
sf1616	Oil	n	R	Germany	S_5 of Baso(cms) × MOL-Rh
sf1636	Oil	n	R	Germany	BC_1S_4 of Baso(cms) × MOL-Rh

AFLP analyses

AFLP analyses using *Eco*RI primers E32, E33, E38, E39, E41, E42, E43 and E46, and *Mse*I primers M47, M48, M49, M59, M61 and M62, were performed according to Vos et al. (1995). For the selective amplification, *Eco*RI primers (500 ng) with three selective nucleotides were labelled using T4 polynucleotide kinase and [γ-^{33}P]-ATP. Labelling was performed for 1 h at 37 °C in 50 µl of kinase buffer (70 mM of Tris/HCl pH 7.6, 10 mM of $MgCl_2$, 100 mM of KCl and 1 mM of 2-mercaptoethanol). Heating the reaction mix to 70 °C for 10 min inactivated the enzyme. For the selective amplification, 5 ng of labelled *Eco*RI primer and 30.2 ng of *Mse*I primer were employed. Alternatively, IRD-labelled *Eco*RI primers from MWG Biotech (Ebersberg, Germany) were used for non-radioactive labelling of the selective amplification products and run on a LICOR (MWG Biotech).

RAPD analyses

From the segregating F_2-population of RHA325 × HA342 one bulk of male-sterile and one of fertility restored plants, consisting of ten individuals each, were formed. In addition the parental lines RHA325 and HA342 were included in the investigations. For each PCR reaction 25 ng of DNA were used as a template in 25 µl of reaction volume. Using 1.5 U of Ampli*Taq* polymerase Stoffel-Fragment, 0.3 µM of primer and 0.4 mM of dNTP were added to the reaction buffer containing 10 mM of Tris/HCl pH = 8.3 and 6 mM of $MgCl_2$. DNA amplification was performed in GenAmp System 9600 or the PE-9600 thermocycler for 45 cycles as follows: 1 min at 94 °C (denaturing), 1 min at 36–58 °C (annealing temperatures were varied depending on the primer) and 2 min at 72 °C (polymerisation). Ramping between annealing and polymerisation was limited to 5 °C (modified according to Sobral and Honeycutt 1993). A total of 520 arbitrary decamer primers (Operon Technologies, kits A to Z) was analysed for the ability to produce polymorphic bands between the bulks.

Conversion of RAPD and AFLP markers into SCAR markers

Bands of RAPD markers were excised from the agarose gel, extracted by the gel-extraction kit and used in the TOPO A Cloning Kit according to the manufacturer's recommendations.

For AFLP markers, a piece of the dried gel corresponding to the marker of interest was cut out and 100 µl of H_2O was added. After 10 min at room temperature samples were boiled for 10 min, centrifuged, and 5 µl of the supernant was used for PCR according to the AFLP conditions. The PCR programme was elongated by an additional final elongation step for 10 min at 72 °C as recommended by the manufacturer of the TOPO A Cloning kit (InVitrogen, Groningen, The Netherlands). Cloning was performed as described in the manual. White clones were picked for overnight culture and for minipreparation of plasmid DNA, using the Qiagen kit. Cloning of the PCR fragment was confirmed by restriction digestion of the clones. The sequence of the cloned PCR amplification product was used to design SCAR primers. PCR amplification was performed in a Perkin Elmer 9600 thermocycler using 50 ng of DNA and 0.2 U of *Taq* polymerase with an initial denaturing step of 10 min at 94 °C, followed by 35 cycles of 45 s at 94 °C, 45 s at T_A (see Table 2) and 60 s at 72 °C, and a final extension step of 6 min at 72 °C, followed by a hold at 8 °C.

Linkage analyses

Linkage analyses were performed using the programme MapMaker Version 3.0b (Lander et al. 1987). The linkage map was constructed on a minimum LOD score of 3.0. The ripple function was used to confirm the linkage map. The Kosambi function was used to obtain the genetic distances in centiMorgan (cM) Kosambi (1944).

Results

Fertility restoration in the presence of PET1

Segregating progeny of a cross between the restorer line RHA325 (PET1 cytoplasm) and the maintainer line HA342 were evaluated for male fertility/sterility. Only two phenotypes were observed in the F_2: male-fertile plants showing normal anthers producing large amounts of yellow pollen and male-sterile plants showing very small anthers but with no pollen. A segregation ratio of 1 (male fertile, *Rf1Rf1*):2 (male fertile, *Rf1rf1*):1 (male sterile, *rf1rf1*) with $\chi^2 = 2.83$ ($P = 0.24$) was observed in the F_2 population (183 individuals) as expected for one restorer gene. In the F_3, 14 plants of each fertile F_2 individual were evaluated for male fertility to be able to distinguish between F_2 individuals being homozygous or heterozygous for the restorer gene *Rf1*. The data obtained for the F_3 populations were confirmed by segregation analyses in the F_2BC_1 populations.

Identification of RAPD and AFLP markers linked to the restorer gene *Rf1*

One bulk of male-sterile and homozygous fertility restored plants consisting of ten plants each were used in the analysis of 520 decamer primers in addition to the two parental lines RHA325 and HA342. Five of the Operon-primers showed polymorphisms between the bulks that could be mapped in the same linkage group as the restorer gene *Rf1*. The size of the polymorphic DNA amplification product is given following the primer name. Three primers OPK13, OPW03 and OPY10 amplified one polymorphic DNA fragment (OPK13_454, OPW03_280 and OPY10_740, respectively) linked in attraction to the restorer gene *Rf1*. OPK13_454 mapped 0.8 cM from *Rf1* in a population of 183 individuals (Fig. 1), followed by OPY10_740 with 2 cM. The two other primers, OPW10 and OPT06, produced DNA markers, OPW10_750 and OPT06_350, linked in repulsion to the restorer gene, which mapped further away from the gene (9.9 cM and 12.3 cM, respectively).

Bulked-segregant analyses using 48 AFLP primer combinations identified 17 markers, which could be mapped in the same linkage group as the restorer gene *Rf1*. E33M61_136, and E41M48_113 were mapped 0.3 cM and 1.6 cM from the gene, respectively. E41M48_113 represented the closest marker linked in attraction to the restorer gene that flanked the gene on the opposite side as the RAPD markers OPK13_454 and OPY10_740 (Fig. 1). The primer combination E39M48 resulted in two markers, E39M48_58 and E39M48_210, that mapped on both sides of the restorer gene. E39M48_58 is the closest AFLP-marker on the same side as the RAPD markers OPK13_454 and OPY10_740, mapping 4.6 cM from the *Rf1* gene. E39M48_210 mapped on the opposite side with 4.4 cM.

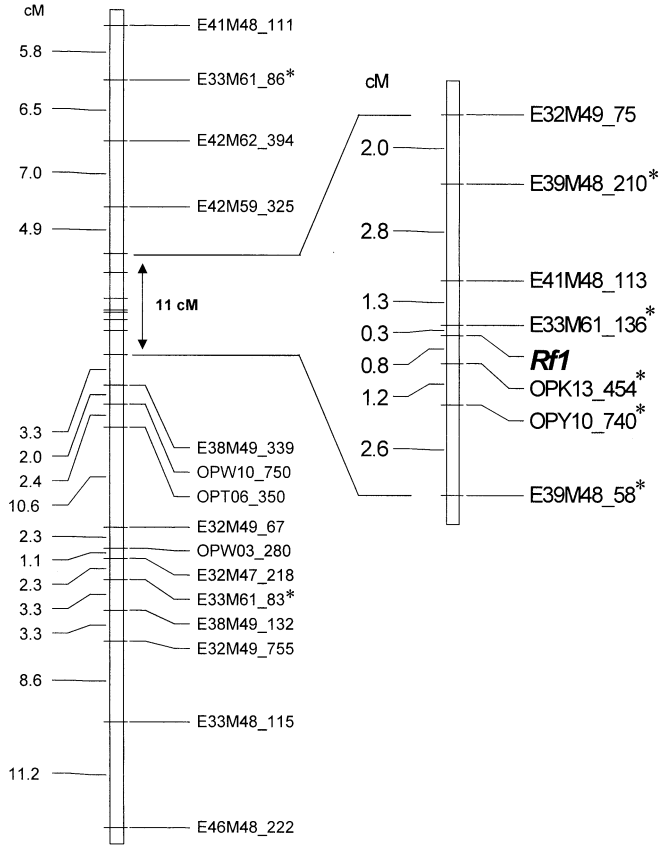

Fig. 1 Linkage map of the restorer gene *Rf1* in sunflower. RAPD and AFLP markers were mapped in the F_2 population of the cross RHA325 × HA342. A region of 11 cM around the restorer gene *Rf1* is shown enlarged. Markers labelled with an *asterix* were also screened in a set of 20 restorer and maintainer lines (see Table 3)

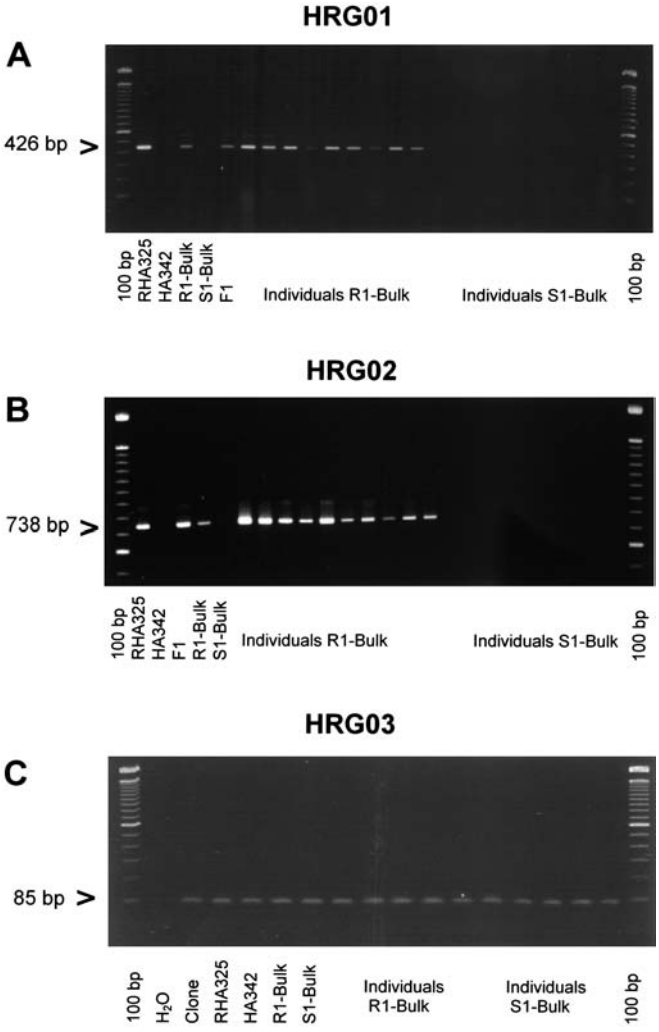

Fig. 2A–C Conversion of RAPD markers into SCAR markers. **A** Marker OPK13_454 converted into SCAR marker HRG01 (426 bp); **B** Marker OPY10_740 converted into SCAR marker HRG02 (738 bp). **C** Marker E41M48_113 converted into the SCAR marker HRG03 (85 bp). Primer sequences are presented in Table 2

As the primer combination E39M48 results in one marker in attraction (E39M48_210) and one in repulsion (E39M48_58) it also allows differentiating heterozygous from homozygous fertility restored plants in segregating populations.

In total, the whole linkage group carrying the restorer gene *Rf1* consists of 17 AFLP markers and five RAPD markers covering 85.4 cM (Fig. 1). This gives an average marker distance of 3.9 cM for this linkage group and a marker density of 1.6 cM in an area of 11 cM around the restorer gene.

Conversion of AFLP and RAPD markers into SCAR markers

For the closest markers flanking the restorer gene *Rf1* in attraction, sequence-specific primers were developed

Table 2 Sequences of SCAR primers derived from RAPD and AFLP markers showing polymorphisms between the bulks of male-sterile and fertility restored F_2 individuals of the cross RHA325 × HA342. Underlined nucleotides are derived from RAPD primers

SCAR-marker	Initial fragment	Primer	Primer sequence	T_A	Expected size of the SCAR-marker	Polymorphism RHA325/HA342
HRG01	OPK13_454	K13spec forward	5′-TAT GCA TAA TTA GTT ATA CCC-3′	58 °C	426 bp	Dominant
		K13spec reverse	5′-ACA TAA GGA TTA TGT ACG GG-3′			
HRG02	OPY10_740	Y10spec forward	5′-AAA CGT GGG AGA GAG GTG G-3′	65 °C	738 bp	Dominant
		Y10spec reverse	5′-AAA CGT GGG CTG AAG AAC TA-3′			
HRG03	E41M48_113	F1SE41M48	5′-TAA CAC GTG CCC TCT GTA-3′	53 °C	85 bp	None
		R1SE41M48	5′-CAG GGG TAT TTA GGG AAC AA-3′			

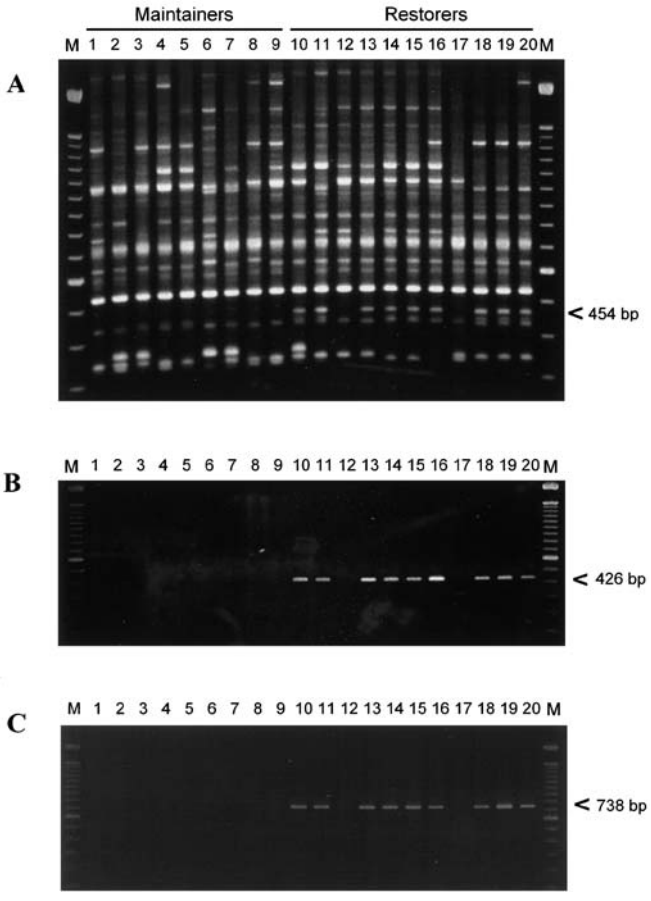

Fig. 3A–C PCR patterns of nine maintainer and 11 restorer lines of the PET1 cytoplasm which were obtained by PCR amplification using the decamer primer OPK13 and the SCAR markers HRG01 and HRG02. **A** Amplification patterns of the primer OPK13. OPK13_454 is marked by an *arrow head*. **B** Amplification patterns using the SCAR-marker HRG01. **C** PCR products obtained using the SCAR marker HRG02. Maintainer lines: *1*, HA342; *2*, HA89; *3*, HA291; *4*, HA323; *5*, HA350; *6*, HA850; *7*, CM594; *8*, CM603; *9*, CM611; Restorer lines: *10*, RHA325; *11*, RHA265; *12*, RHA348; *13*, CM587; *14*, CM592; *15*, CM596; *16*, CM610; *17*, Gio55; *18*, sf1578; *19*, sf1616; *20*, sf1639

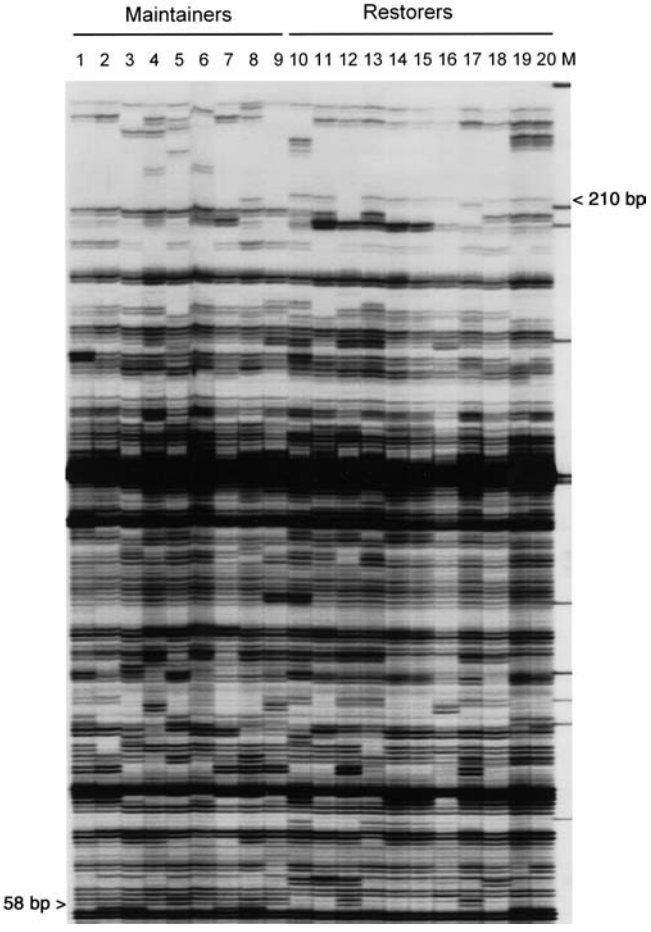

Fig. 4 Comparison of the AFLP patterns (primer combination E39M48) for different restorer and maintainer lines. Maintainer lines: *1*, HA342; *2*, HA89; *3*, HA291; *4*, HA323; *5*, HA350; *6*, HA850; *7*, CM594; *8*, CM603; *9*, CM611. Restorer lines: *10*, RHA325; *11*, RHA265; *12*, RHA348; *13*, CM587; *14*, CM592; *15*, CM596; *16*, CM610; *17*, Gio55; *18*, sf1578; *19*, sf1616; *20*, sf1639

which can be easier handled in backcross programmes (Table 2). Both RAPD markers, OPK13_454 and OPY10_740, were successfully converted into the SCAR markers HRG01 and HRG02 giving amplification products of 426 bp and 738 bp, respectively (Fig. 2). In addition, the closest AFLP-marker in attraction was cloned and sequenced. However, the sequence-specific primers derived from the AFLP marker E41M48_113 only gave a monomorphic pattern (Fig. 2).

Utility of the identified markers in other cross combinations

To investigate the utility of these closely linked markers to the *Rf1* gene in marker-assisted selection, a set of 20 restorer and maintainer lines was screened with these markers (Fig. 3). A comparison of the patterns obtained for 11 restorer lines and nine maintainer lines by using OPK13_454, or the corresponding primers for the SCAR marker HRG01, demonstrated that none of the maintainer lines showed an amplification product of the expected size with these primers whereas all investigated restorer lines, except the high oleic line RHA348 and the dwarf line Gio55, gave the corresponding amplification product. The investigated restorer lines also included three lines developed from the interspecific hybrids *H. annuus* × *Helianthus mollis* and *H. annuus* × *Helianthus rigidus* (Table 1). The same results were obtained for HRG02. This demonstrates that both SCAR markers HRG01 and HRG02, tightly linked to the restorer gene *Rf1*, can also be used in the development of new sunflower lines derived from interspecific hybrids. HRG01 and HRG02 are dominant markers that can be easily applied in marker-assisted programmes for the development of new restorer or maintainer lines. However, due to the dominant

Table 3 Screening of 20 restorer and maintainer lines of the PET1 cytoplasm in sunflower using SCAR and AFLP markers linked to the restorer gene *Rf1*

Line	HRG01	HRG02	E33M61_136	E33M61_86	E33M61_83	E39M48_58	E39M48_210
HA342	–	–	+	+	+	+	–
HA89	–	–	–	+	–	+	–
HA291	–	–	–	–	+	+	–
HA323	–	–	–	–	+	+	–
HA350	–	–	+	+	–	+	–
HA850	–	–	–	–	–	+	–
CM594	–	–	–	–	–	+	–
CM603	–	–	nd*	nd	nd	+	–
CM611	–	–	–	–	–	+	–
RHA325	+	+	–	–	–	–	+
RHA265	+	+	–	–	–	–	+
RHA348	–	–	+	+	+	+	–
CM587	+	+	nd	nd	nd	–	+
CM592	+	+	nd	nd	nd	–	+
CM596	+	+	–	–	–	–	+
CM610	+	+	+	–	–	–	+
Gio55	–	–	–	–	–	+	–
sf1578	+	+	–	–	–	–	+
sf1616	+	+	–	–	–	–	+
sf1636	+	+	–	–	–	–	+

* nd, not determined

scoring plants heterozygous or homozygous for the restorer gene *Rf1* cannot be distinguished.

The primer combination E39M48, which gives markers in repulsion (E39M48_58) and attraction (E39M48_210) to the *Rf1* gene, was tested in the same set of 20 restorer and maintainer lines (Fig. 4). E39M48_58 was amplified in all maintainer lines, but not in the restorer lines except RHA348 and Gio55. E39M48_210 was present in all restorer lines except RHA348 and Gio55, but not in any of the investigated maintainer lines. The three restorer lines developed from the interspecific hybrids *H. annuus* × *H. mollis* and *H. annuus* × *H. rigidus* (Table 1) showed the same amplification product. Therefore, this primer combination, which allows distinguishing heterozygous and homozygous fertility restored plants, can be used in a large number of cross combinations including interspecific crosses. An overview about all markers tested in the set of 20 maintainer and restorer lines is given in Table 3.

Discussion

Marker-assisted selection (MAS) is especially relevant in breeding programmes if the trait of interest, like e.g. fertility/sterility or yield, can only be evaluated after flowering. Bulked-segregant analyses according to Michelmore et al. (1991) have been successfully used to identify markers tightly linked to genes that are responsible for fertility restoration in petunia, rye, broad bean and rice (He et al. 1995; Zhang et al. 1997; Bentolila et al. 1998; Börner et al. 1998). In petunia, fine mapping has even identified an AFLP marker cosegregating with the restorer of fertility (*Rf*) locus (Bentolila and Hanson 2001). For sunflower, the two identified PCR-based markers, OPK13_454 and OPY10_740, are very interesting for marker-assisted selection as they map within less than 2 cM from the restorer gene *Rf1*. The conversion of OPK13_454 and OPY10_740 into SCAR markers (HRG01 and HRG02) makes them even more valuable as they are easier to handle. In addition, both SCAR markers, HRG01 and HRG02, proved to be nearly universally usable in crosses between restorer and maintainer lines. In contrast, the sequence-specific primers derived from the AFLP marker E41M48_113 only gave a monomorphic pattern, probably due to its small amplification product of 85 bp. However, the sequence of the AFLP marker E41M48_113 can be used to construct an overgo probe and to screen a genomic library for a map-based cloning approach. Although AFLP represents a more-reliable and reproducible marker technology than RAPD, the possibility of converting AFLP markers into sequence-specific markers is often restricted due to the very small size of the markers and the fact that most AFLP polymorphisms seem to originate in differences within the restriction sites (Meksem et al. 1995; Shan et al. 1999).

To evaluate the broad utility of the two SCAR markers, HRG01 and HRG02, the set of 20 investigated lines also included three restorer lines developed from the interspecific hybrids *H. annuus* × *H. mollis* and *H. annuus* × *H. rigidus*. These restorer lines gave amplification products of the expected size by using the two SCAR markers, which indicates that the restorer gene *Rf1* seems also to be present in these wild species. Therefore, also from the wild species, introduction of interesting traits like disease resistances can be accompanied by marker-assisted selection for the restorer gene using these two sequence-specific markers. With regard to mapping restorer genes from interspecific crosses, only

the restorer gene *Rf1*-PEF1 and the additional genetic factors controlling pollen viability have so far been localised in the backcross *H. annuus* cv RHA274 × (*Helianthus argophyllus* No. 92 × *H. annuus* cv RHA274) using RAPD markers and isozymes (Quillet et al. 1995). It would be interesting to investigate whether restorer genes of different CMS sources in sunflower, like e.g. PEF1, PET2 and ANN4 (Horn and Friedt 1999; De la Canal et al. 2001; Horn 2002), for which restorer lines have been identified (Quillet et al. 1995; Horn and Friedt 1997), cluster together or represent alleles of one gene as described for *Fr2*, $Fr_{PI207228}$ and Fr_{XR235} in *Phaseolus vulgaris* (Jia et al. 1997), or whether they are unlinked or even located on different chromosomes. This requires identification of markers closely linked to these restorer genes and crosses between the different restorer lines.

In the presented study we identified RADP and AFLP markers closely linked to the restorer gene *Rf1* in sunflower, which restores fertility in presence of the PET1 cytoplasm. Conversion of two RAPD markers into SCAR markers resulted in markers easy to be handled in marker-assisted selection. Their broad utility, especially also in inbred lines derived from interspecific hybrids, makes them a valuable tool for hybrid breeding in sunflower. With these SCAR markers in hand, development of new restorer and maintainer lines for sunflower hybrid breeding can now be performed much more efficiently.

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