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ORIGINAL PAPER

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Identification of SCAR markers linked to *Rca2* anthracnose resistance gene and their assessment in strawberry germplasm

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Abstract Bulked segregant analysis combined with AFLPs was used to identify molecular markers linked to the *Rca2* gene conferring resistance to *Colletotrichum acutatum* pathogenicity group 2 which causes anthracnose in the octoploid strawberry *Fragaria* × *ananassa*. DNA bulks originating from a cross between the resistant cultivar 'Capitola' and the susceptible cultivar 'Pajaro' were screened with 110 *Eco*RI/*Mse*I AFLP combinations. Four AFLP markers were found linked in coupling phase to *Rca2* with recombination percentages between 0% and 17.7%. Among the four markers linked to the resistance gene, two were converted into SCAR markers (STS-Rca2_417 and STS-Rca2_240) and screened in a large segregating population including 179 genotypes. The *Rca2* resistance gene was estimated to be 0.6 cM from STS-Rca2_417 and 2.8 cM from STS-Rca2_240. The presence/absence of the two SCAR markers was further studied in 43 cultivars of *F.* × *ananassa*, including 14 susceptible, 28 resistant, and one intermediate genotype. Results showed that 81.4% and 62.8% of the resistant/susceptible genotypes were correctly predicted by using STS-Rca2_417 and STS-Rca2_240, respectively. The 14 susceptible genotypes showed no amplification for either SCARs. These developed SCARs constitute new tools for indirect selection criteria of anthracnose resistance genotypes in strawberry breeding programs.

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Introduction

Three fungal species have been reported as causal agents of strawberry (*Fragaria* × *ananassa*) anthracnose: *Colletotrichum acutatum* J.H. Simmonds, *C. fragariae* Brooks, and *C. gloeosporioides* (Penz.) Penz. & Sacc. (teleomorph, *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk) (Howard et al. 1992). In Europe, anthracnose is most often incited by *C. acutatum*. While anthracnose infections may occur on all parts of the strawberry plant, symptoms most commonly occur on runners, flowers, and fruits. Cultural practices, such as the use of pathogen-free plants produced according to a scheme of certification (Nourrisseau 1986; OEPP/EPPO 1994) and plastic tunnels, have led to considerable reduction of losses in field production. However, severe outbreaks still occur in nursery beds. Moreover, latent infection in apparently healthy plants may lead to anthracnose symptoms in production fields (McInnes et al. 1992). Therefore, the development of strawberry cultivars resistant to *C. acutatum* is necessary for anthracnose control.

Different modes of inheritance for resistance to anthracnose have been suggested based on the pathogenicity groups of *C. acutatum* (Denoyes and Baudry 1995). Resistance to pathogenicity group 1 is quantitative, whereas a single dominant gene, *Rca2*, controls the resistance to pathogenicity group 2 although minor genes may also contribute to this resistance in several cultivars (Denoyes-Rothan et al. 2005).

Molecular markers linked to resistance genes can be useful in breeding programs (Marker-Assisted Selection, MAS) to help select for more resistant cultivars. Bulked segregant analysis (BSA) (Michelmore et al. 1991) proved to be effective for detecting markers linked to resistance genes in several polyploid species, including

cassava (Akano et al. 2002), roses (von Malek et al. 2000), and cultivated strawberry ($2n = 8x = 56$) (Haymes et al. 1997).

The objectives of the present work were to develop molecular markers linked to the *Rca2* locus by using the BSA approach associated with AFLP markers and to evaluate their association with anthracnose resistance in strawberry germplasm.

Materials and methods

Plant material

In order to detect markers linked to the *Rca2* gene, a F_1-family of 179 individuals from the cross 'Capitola' × 'Pajaro' was analysed. Both female ('Capitola': 'CA 75.121.101'×'Parker') and male parents ('Pajaro': 'Sequoia'×'Cal 63.7-101') are selections from University of California (Davis, USA). The cultivar 'Capitola' is resistant to *C. acutatum* and displays a heterozygous dominant gene (*Rca2*/*rca2*) conferring resistance to isolates of pathogenicity group 2 (Denoyes-Rothan et al. 2005). The cultivar 'Pajaro' is susceptible to *C. acutatum* and is recessive homozygous (*rca2*/*rca2*) for this resistant gene. For genotyping resistance to anthacnose of the 179 F_1-individuals, we developed 179 F_1S_1-families derived from the selfing of each F_1-individual. Due to losses stemming from inbreeding, the number of S_1 seedlings obtained from each of the 179 F_1-progeny varied from 17 to 128 with a mean value of 44. Forty-three accessions reported in the literature as resistant or susceptible to anthracnose (Table 1) were tested for presence of the developed SCAR markers.

Progeny genotyping of resistance to anthracnose, pathogenicity group 2

Seedlings were inoculated 6–8 weeks after germination by spraying the leaf surfaces until runoff with a conidial suspension adjusted to 3×10^6 conidia/ml (Denoyes-Rothan et al. 2005). A mono-conidial isolate of *C. acutatum* belonging to pathogenicity group 2, either 1267b or 688b, was used as inoculum. After inoculation, the seedlings were maintained in a controlled environment chamber.

Scoring of the disease was done 28 days after inoculation on a scale from 0 (no visual symptoms) to 5 (death of the plant) (Denoyes and Baudry 1995). The S_1 of the two parents, 'Capitola' and 'Pajaro', were included as controls in inoculation tests. In order to study the segregation of the resistance allele, seedlings were grouped into two classes based on their disease scores: the resistant class (R) (disease scores 0 and 0.5, which represented no developed visual symptom) and the susceptible class (S) (disease scores from 1 to 5 corresponding to petiolar and/or foliar necrosis and/or wilting).

Segregation ratios of the 179 F_1S_1 families were tested for goodness-of-fit to theoretical ratios for resistance controlled by one dominant allele. These F_1S_1-families displayed segregation ratios of 3:1 (resistant:susceptible) for the *Rca2*/*rca2* F_1-families and 0:1 for the *rca2*/*rca2* F_1-families. Since seedlings may escape inoculation (mean of 3% in the susceptible S_1 cultivars in Denoyes-Rothan et al. (2005)), all the segregation ratios 0:1 (resistant:susceptible) were tested as 3:97 (resistant:susceptible). Tests were performed on the segregating populations using numerical data. The chi-square (χ^2) test was used for testing the segregation ratio 3:1. The likelihood ratio chi-square test (G^2) (SAS Institute, Inc., Cary, NC, USA), which can be performed using a low number of observations (Arbonnier 1966), was used for the segregation ratios 0:1 (tested as 3:97). The significance level was considered at $P = 0.01$.

AFLPs and bulk composition

About 90 mg of young leaves were ground in liquid nitrogen and DNA was extracted using a DNeasy Plant Mini Kit from Qiagen (Courtaboeuf, France). AFLP analysis was performed according to Vos et al. (1995) and modified as in Lerceteau-Köhler et al. (2003) using primers containing one selective nucleotide (*Eco*RI + A and *Mse*I + C) for the first selective amplification products and primers containing three selective nucleotides (*Eco*RI + 3/*Mse*I + 3) for the second amplification. Two resistant and two susceptible bulks (B_{R1},B_{R2} and B_{S1}, B_{S2}, respectively) were constructed of equal aliquots of the first selective amplification products from ten resistant or ten susceptible F_1-plants for each bulk. The 20 susceptible and 20 resistant F_1-plants were chosen on the basis of the results of χ^2 or G^2 test in their corresponding F_1S_1-family. The 40 F_1S_1-families showed segregations consistent with a 3:1 ratio indicating a resistant F_1-plant (*Rca2*/*rca2*), or with a 0:1 ratio indicating a susceptible F_1-plant (*rca2*/*rca2*). The bulking of the pre-amplified DNA instead of the native DNA eliminated problems associated with poor quality DNA or partial digestion, which render difficult the identification of linked fragments (Dong et al. 2000). Putative markers were further validated by separate analysis of the ten F_1-individuals constituting each bulk.

SCAR design and analysis

The AFLP fragments potentially associated with resistance were excised from the dried polyacrylamide gel, re-hydrated in TE buffer for 2 h at 56 °C and briefly centrifuged. One and a half µl of the supernatant was used as template for PCR amplification using primers and reaction conditions similar to those used for the AFLP reaction. The amplified DNA was cloned into the pGEM-T plasmid vector (Promega, Charbonnières, France) according to manufacturer's specifications.

PCR products of the cloned fragments were checked on the sequencing gel to ensure that the insert sizes corresponded to the size of the AFLP fragments cloned. The cloned DNA fragments presenting the appropriate size were sequenced, and primers were designed from the sequence data.

The SCAR marker STS-Rca2_240 was multiplexed with the *Fragaria vesca* microsatellite EMFv020 (Hadonou et al. 2004) used as the positive PCR control. The amplification procedure was carried out in total volume of 15 µl with 1x reaction buffer (Sigma-Aldrich, St Quentin Fallavier, France), 0.2 mM of each dNTP, 0.2 µM of each primer CAC_240_2F, CAC_240_2Rb, EMFv020_F and EMFv020_R, 1.5 mM $MgCl_2$, 0.8 U Taq polymerase (Sigma-Aldrich, St Quentin Fallavier, France) and 16 ng of DNA. The reaction consisted of

Table 1 Genotype and cultivar names, their parents, resistance phenotype or genotype, and their status for the SCAR markers (+ present; - absent)

Genotype	Parents	Origin	Resistance geno/phenotype	STS-Rca2_417	STS-Rca2_240
'Addie'	'S. Pantagruella' × 'MDUS 3816'	Italy	I[a]	–	–
'Aïko'	'Cal 46.5-1' × 'Cal 59.51-11'	USA (CA)	RR or Rr[b]	+	+
'Arking'	'Cardinal' × 'Ark 5431' ('MDUS 3082' × 'Delite')	USA (FL)	Rr[a]	–	–
'Bogota'	('Climax' × 'Deutsch Evern') × 'Tago Zb.53.116' × 'Tago'	NL	rr[a]	–	–
'Brighton'	'65.65-601' (complex parentage) × 'Tufts'	USA (CA)	rr[b]	–	–
'Capitola'	'CA 75.121.101' × 'Parker'	USA (CA)	Rr[a]	+	+
'CF1116'	'Pajaro' × 'CF129'	France	rr[a]	–	–
'CF129'	'Earlyglow' × 'Chandler'	France	rr[a]	–	–
'Chandler'	'Douglas' × 'Cal 72.361-105'	USA (CA)	Rr[a]	+	+
'Cruz'	'Cal 37.20-45' × 'Sequoia'	USA (CA)	Rr[b]	+	–
'Darselect'	'Elsanta' × 'Parker'	France	rr[a]	–	–
'Darsidor'	'Seducta' × 'Aiko'	France	Rr[c]	+	–
'Dover'	'Florida Belle' × 'USFL 71-189'	USA (FL)	RR[a,b]	+	+
'Earlyglow'	'Fairland' × 'Midland') × ('Redglow' × 'Surecrop')	USA (MD)	rr[a]	–	–
'Elsanta'	'Gorella' × 'Holiday'	Netherlands	rr[a,b]	–	–
'Gariguette'	('Pocahontas' × 'Regina') × ('Belrubi' × 'Marieva')	France	Rr[a]	+	–
'Hecker'	'CA 69.96-101' × 'CA 65.65-601'	USA (CA)	rr[b]	–	–
'Hokowase'	'Yakumo' × 'Tahoe'	Japan	rr[a]	–	–
'Mamie'	'Harwester' × 'Gariguette'	France	Rr[a]	+	–
'Mara des Bois'	('HummiGento' × 'Ostara') × ('Redgauntlet' × 'Korona')	France	Rr[a]	–	–
'Mme Moutot'	'Dr Morère' × 'Royal Sovereign'	France	Rr[a]	–	–
'Oso Grande'	'Parker' × ('Tioga' × 'Pajaro')	USA (CA)	Rr[a]	+	+
'Pajaro'	'Sequoia' × 'Cal 63.7-101'	USA (CA)	rr[a,b]	–	–
'Parker'	'Douglas' × ('Tufts' × 'Cal 63.7.101')	USA (CA)	Rr[b]	+	+
'Redchief'	'NC 1768' × 'Surecrop'	USA (MD)	rr[b]	–	–
'Revada'	'Climax' × 'Ada Herzberg'	NL	Rr[a]	+	–
'Rosanne'	'NC 3140' × self	USA (MD)	Rr[a]	–	–
'Salinas'	'Cal 37.2-11' × 'Cal 42.17-18'	USA (CA)	RR or Rr[b]	+	–
'Scott'	'Sunrise' × 'Tioga'	USA (MD)	RR or Rr[b]	+	+
'Seascape'	'Selva' × 'Douglas'	USA (CA)	Rr[a]	+	+
'Selva'	'CA 70.3-177' × 'CA 71.98-605'	USA (CA)	Rr[a]	+	+
'Senga Sengana'	'Markee' × 'Sieger'	GER	Rr[a]	–	–
'Sequoia'	'Cal 52.16-15' × 'Cal 51s1-1'	USA (CA)	Rr[a,b]	+	–
'Soquel'	'Cruz' × 'Aiko'	USA (CA)	RR or Rr[b]	+	+
'Surecrop'	'Fairland' × 'USMD 1972'	USA (MD)	rr[b]	–	–
'Tioga'	'Lassen' × 'Cal 42.8-16'	USA (CA)	Rr[b]	+	+
'Tufts'	'CA 46.5-1' × 'Tioga'	USA (CA)	Rr[b]	+	+
'US159'	'LA 7525-A' × 'US 78-1760 AN'	USA (MS)	RR[a]	+	+
'US292'	'Arking' × 'LA 883'	USA (MS)	RR[a]	–	–
'US438'	'MSUS 56' × 'MSUS 70'	USA (MS)	Rr[a]	–	–
'US70'	'FL 76-802' × 'LA 2556'	USA (MS)	Rr[a]	+	–
'Valeta'	'Sivetta' × 'Holiday'	Netherlands	rr[a]	–	–
'Yolo'	'Hecker' × ('Tufts'x'Cal 63.7-101'	USA (CA)	rr[a,b]	–	–

Names were checked according to the National Clonal Germplasm Repository at Corvallis (USA) and the ISHS strawberry group (http://www.ars-grin.gov/ars/PacWest/Corvallis/ncgr/; http://www.agraria.it/isf/attivit/fragola/ishs/varieties.htm)
Genotypes and phenotypes were described according to previous studies:

[a]Denoyes-Rothan et al. (2005)
[b]Winterbottom (1991)
[c]Denoyes-Rothan, unpublished results. *RR* Homozygous resistant; *Rr* heterozygous resistant; *rr* homozygous susceptible. *I* Phenotype of intermediate resistance

Table 2 AFLP, SCAR and primer denotations, primers sequences, annealing temperature for SCAR and control PCR amplifications, and type of polymorphism

AFLP marker	SCAR name	Primer name	Primer sequences	Annealing temperature	Polymorphism
aga/cac_280	STS-Rca2_240	CAC_240_2F	5′-GCC ACG TCA CTA GTC AAA TTC AA-3′	64°C	Dominant
		CAC_240_2RB	5′-TCA TGG ACA GTG GTC TCA GC-3′		
aga/cac_488	STS-Rca2_417	CAC_417_3F	5′-ACC ATG CAG AAC GTT CAG ATA T-3′	62°C	Dominant
		CAC_417_3R	5′-TCC CAG CTG AAG ATC AAT GTA GT-3′		
–	Control PCR	EMFv020_F[a]	5′-CAG GCG CCA ACG GCG TGC TCT TGT-3′	64°C	–
		EMFv020_R[a]	5′-CAG CGC CGC CAG CTC ATC CCT AGG –3′		

[a]Primers from *F. vesca* microsatellite (Hadonou et al. 2004)

3 min denaturation at 95°C, 35 cycles of 50 s at 95°C, 50 s at 64°C, and 1 min at 72°C, and a final extension step of 5 min at 72°C. The amplification product was separated on a 2% agarose gel.

The amplification procedure of the SCAR marker STS-Rca2_417 was carried out in the same way as for STS-Rca2_240 except that the forward primer CAC_417_3F was previously labelled with [$\gamma-^{33}$ P] ATP. The amplification conditions were similar to the ones used for STS-Rca2_240 with the exception of the annealing temperature at 62°C. The amplification product was separated on a 4.5% denaturing polyacrylamide gel at 95 W for 3 h. Primer sequences are reported in Table 2.

Linkage analysis

Segregation ratios of the putative linked AFLP and SCAR markers were tested for goodness-of-fit to theoretical ratios of 1:1, expected for simplex markers that differ between the two parents (i.e. presence in one and absence in the other), whatever the chromosome pairing (Wu et al. 1992). The significance level was considered at $P = 0.01$ as described above. Linkage analysis was performed using the MAPMAKER/EXP 3.0 (Lander et al. 1987). The markers were assigned to the linkage groups using a LOD≥3.0 and a recombination frequency of $\theta \leq 0.4$. The Kosambi mapping function (Kosambi 1944) was used to convert the recombination fractions into map distances. These SCAR markers were also mapped in our reference population obtained from the cross 'Capitola'×'CF1116' (Lerceteau-Köhler et al. 2003).

Results

Genotypic characterisation of the resistance

Ninety-six F_1S_1-families and the S_1-family of the resistant parent, 'Capitola', segregated clearly in a 3:1 (resistant:susceptible) ratio ($P > 0.01$) (Table 3). Eighty-three F_1S_1-families and the S_1-family of the susceptible parent, 'Pajaro', showed a low percentage of resistant seedlings (mean of 5%) and their segregation ratio supported the absence of dominant allele (0:1 segregation ratio, $P \geq 0.01$). Therefore, within the F_1-family, 96 individuals were *Rca2*/*rca2* and 83 individuals *rca2*/*rca2*, which fitted the 1:1 expected ratio ($P = 0.33$) corresponding to one dominant allele controlling the high level of anthracnose resistance to pathogenicity group 2. Examples of the disease score distribution in four F_1S_1-families and in the two parental S_1-families are presented in Fig. 1. Most of the seedlings were scored 0 (no

Table 3 Segregation ratios of the 'Capitola' (resistant) and 'Pajaro' (susceptible) parental S_1 families, of the 179 F_1S_1 ('Capitola' × 'Pajaro') progeny and of the F_1-progeny according to the F_1S_1-families after inoculation with *Colletotrichum acutatum*, pathogenicity group 2, from strawberry

Populations	No. of plants[a]	Classes[b]		One major resistant gene	P[d]
		[R]	[S]	Ratio[c]	
Capitola-S_1	428	336	92	3:1	0.09
Pajaro-S_1	371	20	351	0:1	0.01
Segregation ratio for the 179 F_1S_1-families					
For 96 F_1S_1-families	18–128 (47)	15–84(34)	3–44(13)	3:1	0.01–1.00
For 83 F_1S_1-families	17–118(40)	0–8(2)	16–110(38)	0:1	0.03–0.98
Segregation ratio for the F_1-progeny according to the F_1S_1-families	179	96	83	1:1	0.33

[a]Total number of S_1 or F_1S_1-inoculated plants. For the F_1S_1-families, numbers indicated the range of all the studied families and the mean is indicated into brackets

[b]Plants scored from 0 to 0.5 are classed as resistant [R], plants scored from 1 to 5 are classed as susceptible [S]. Disease symptoms are scored 28 days after inoculation

[c]Ratios of resistant [R] to susceptible [S] plants

[d]P represented the probability of obtaining deviations from the expected ratio by chance alone. A probability value equal or greater than 0.01 indicates that segregation in the observed population does not differ significantly from the expected ratio

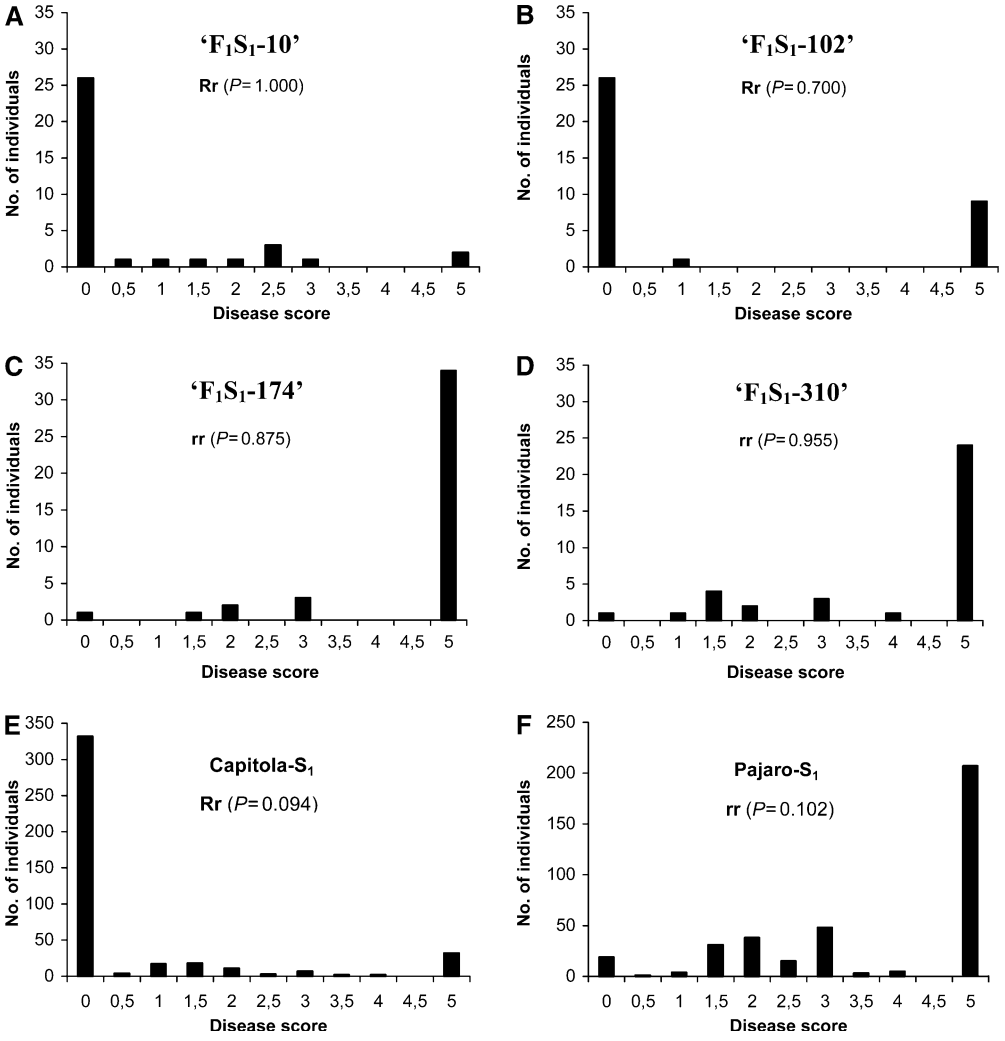

Fig. 1 Distribution of four F_1S_1-strawberry seedling families from the 'Capitola' × 'Pajaro' cross (**a–d**) and the parental S_1-families (**e, f**) according to the disease scores after *Colletotrichum acutatum* (pathogenicity group 2) inoculation. Plants scored from 0 to 0.5 constitute the resistant class [*R*], and plants scored from 1 to 5.0 the susceptible class [*S*]. Genotype (*Rr*, heterozygous dominant, *rr*, homozygous susceptible), and probability (*P*) of chi-square for Rr genotypes or likelihood chi-square for rr genotypes (*in brackets*) are mentioned

symptom) or 5 (dead seedling), which suggested a major gene effect, confirmed by studying the inheritance of *Rca2* (Denoyes-Rothan et al. 2005). However, intermediate disease scores were observed, which could suggest the action of minor genes.

Identification of AFLP markers linked to the *Rca2* gene

A total of 110 *Eco*RI + 3/*Mse*I + 3 combinations were tested on the susceptible and resistant bulks and parents. Per primer combination, the total number of unique clear AFLP bands observed in the two parents varied between 4 and 134 with an average of 56.9. Of the 6,259 amplified fragments, four AFLP markers (aga/cag_320, atc/caa_175, aga/cac_280, aga/cac_488) (0.06% of the fragments) were found to be present in the resistant parent 'Capitola' and both resistant bulks B_{R1} and B_{R2}, whereas they were absent in the susceptible parent 'Pajaro' and both susceptible bulks B_{S1} and B_{S2} (Fig. 2, primer combination *Eco*RI-AGA/*Mse*I-CAC). Recombination frequencies between the 4 AFLP markers and the gene *Rca2* were established using 62 individuals of the F_1-progeny, including the 40 individuals from the bulks (Fig. 2, marker aga/cac_280). Recombination percentages of 17.7, 1.6 and 1.6% were found between *Rca2* and aga/cag_320, atc/caa_175 and aga/cac_280 respectively. No recombination was detected between the resistance allele and the marker aga/cac_488. All the markers were linked in coupling phase with the resistance gene and located on the same side of *Rca2* (Fig. 3).

SCAR development

Two of the four markers (aga/cac_280 and aga/cac_488) linked to the resistance gene were converted into SCAR markers. For the AFLP marker aga/cag_320, sequence-specific primers generated a monomorphic pattern. Due to the large distance between this marker and the resistance gene, this marker was of low interest for future MAS; therefore, no further work, such as sequencing amplified fragments from resistant and susceptible parents, was attempted. The AFLP marker atc/caa_175 was

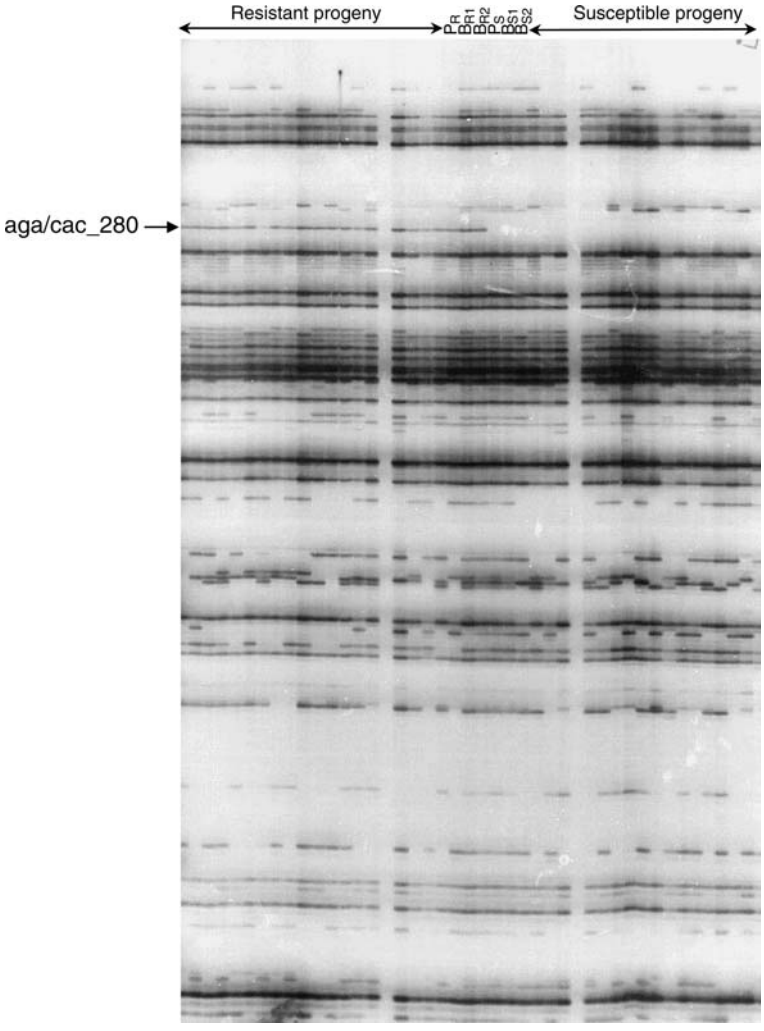

Fig. 2 AFLP gel with the *Eco*RI-AGA/*Mse*I-CAC primer combination. The AFLP marker aga/cac_280 (*arrow*) was found linked to *Colletotrichum acutatum*, pathogenicity group 2 resistance. This marker was present in the resistant bulks, B_{R1} and B_{R2}, and the resistant parent 'Capitola' (P_R), and absent in the susceptible bulks, B_{S1} and B_{S2} and the susceptible parent 'Pajaro' (P_S)

not further analysed since it belonged to a complex of bands, making its conversion probably difficult. In addition, it cosegregated with the marker aga/cac_280.

The AFLP marker aga/cac_280, which displayed a clear and well-isolated band compared to other markers, was transformed into the dominant SCAR marker STS-Rca2_240 (Fig. 4a). This marker was evaluated by multiplexing with the *F. vesca* microsatellite EMFv020. The genetic distance between the STS-Rca2_240 and the resistance gene, evaluated on the 179 F_1-progeny, was 2.8 cM (five recombinants observed).

The transformation of the AFLP marker aga/cac_488, which cosegregated with the resistance gene in the AFLP linkage map, generated several bands separated on a polyacrylamide gel. The size of these bands ranged from 417 bp to 421 bp with an additional band at 397 bp (Fig. 4b). One of them, the SCAR marker STS-Rca2_417, was linked to the resistance gene *Rca2* with a genetic distance of 0.6 cM (one recombinant observed). In the resulting map of the resistance gene and the SCARs STS-Rca2_240 and STS-Rca2_417, which both fitted a 1:1 segregation ratio ($P = 0.16$ and $P = 0.29$ respectively), both SCAR markers were located

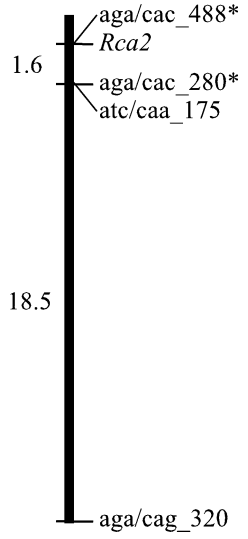

Fig. 3 A genetic map of the chromosome region containing the *Rca2*, *Colletotrichum acutatum*, pathogenicity group 2, resistance gene. The map is based on 62 F_1-individuals from the 'Capitola' × 'Pajaro' cross. AFLP markers labelled with an asterisk (*) were successfully converted into SCAR markers

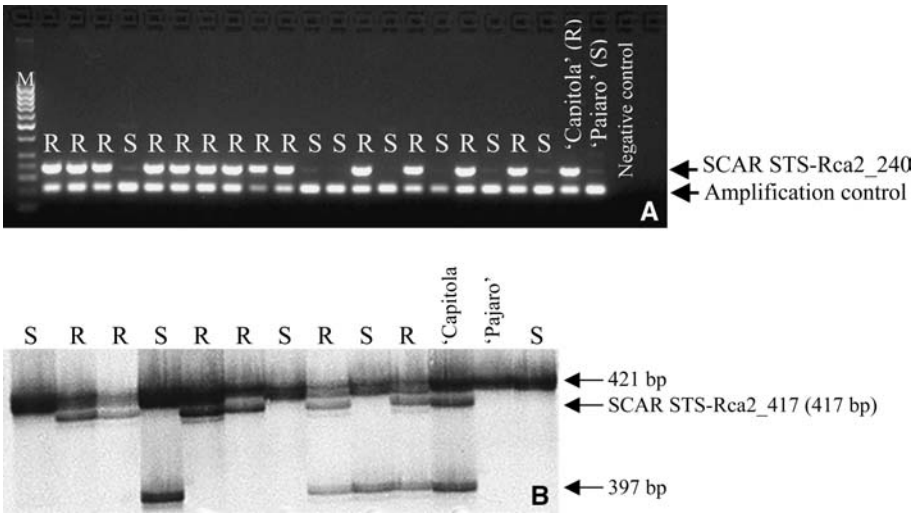

Fig. 4 Detection of the SCAR markers STS-Rca2_240 (**a**) and STS-Rca2_417 (**b**) by PCR amplification and gel electrophoresis of 20 and 11 strawberry individuals, respectively, from the cross between 'Capitola' and 'Pajaro'. *R* Resistant individuals; *S* susceptible individuals. Amplification control corresponds to the amplification of the *Fragaria vesca* microsatellite EMFv020. *M* Molecular weight marker 1 Kb

on the same side with a recombination frequency of 2.2% between them.

Mapping of the SCAR markers in the 'Capitola'×'CF1116' F_1-population

The SCAR markers were added to the 'Capitola'×'CF1116' reference map (Lerceteau-Köhler et al. 2003) which population size was increased to 213 individuals. The AFLP markers from which the SCARs were derived have previously been mapped on linkage group F4 of the female map. This group comprises 17 markers spread 90 cM apart and all linked in coupling phase. As expected, the SCARs cosegregated with their original AFLP markers. The recombination frequency between the two SCARs in this population equalled 1.5%.

Evaluation of strawberry genotypes using SCAR markers

Presence/absence of the two SCAR markers was studied on 43 genotypes or cultivars of *F.* × *ananassa* (Table 1). None of the 14 susceptible genotypes (rr) exhibited the markers. Among the 28 resistant genotypes, 13 (46.4%) amplified both SCARs ('Aïko', 'Capitola', 'Chandler', 'Dover', 'Osogrande', 'Parker', 'Scott', 'Seascape', 'Selva', 'Soquel', 'Tioga', 'Tufts', 'US159'), 8 (28.6%) amplified STS-Rca2_417 but not STS-Rca2_240 ('Cruz', 'Darsidor', 'Gariguette', 'Mamie', 'Revada', 'Salinas', 'Sequoia', 'US70') and 7 (25%) exhibited neither marker ('Arking', 'Mara des Bois', 'Mme Moutot', 'Rosanne', 'Senga Sengana', 'US292', 'US438'). For the intermediate resistant genotype Addie, none of the SCAR markers amplified. None of the resistant genotypes had STS-Rca2_240 without STS-Rca2_417.

Discussion

We successfully characterised four AFLP markers linked to the resistance gene *Rca2* using the BSA strategy. These four markers were confirmed to be linked to the resistance gene, and no false positives were detected. Since the choice of genotypes for the bulks is critical, F_1-progeny were genotyped by inoculating the F_1-S_1-seedlings rather than plants from vegetative multiplication. Using this approach, a clear distinction was obtained between the 3:1 S_1ratio associated with the segregation of the *Rca2*/*rca2* genotypes and the 0:1 S_1 ratio associated with the segregation of the *rca2*/*rca2* genotypes. In addition, using two bulks per anthracnose phenotype instead of one increased the efficiency of the method.

Segregation of both the resistance and linked markers clearly indicated monogenic control of resistance confirming the results reported by Denoyes-Rothan et al. (2005). In the octoploid cultivated strawberry, monogenic control has already been described for several traits such as sex expression (Ahmadi and Bringhurst 1991), photo-insensitivity (day-neutrality) (Ahmadi et al. 1990), and more recently, resistance to *Phytophthora fragariae* (Van de Weg 1997).

Conversion of AFLP markers into SCARs

We converted two of the AFLP markers linked to *Rca2*, aga/cac_280 and aga/cac_488, into SCAR STS-Rca2_240 and STS-Rca2_417, respectively. These markers were dominant and were revealed on agarose (STS-Rca2_240) or on acrylamide gels (STS-Rca2_417) due to presence of a complex of bands including one linked to the resistance gene. With the AFLP marker aga/cag_320, we faced a rather common problem, i.e. the generation of monomorphic patterns (e.g. Prins et al. 2001; Horn et al. 2003). Since most of the AFLP polymorphism originates in

differences within restriction sites, these differences are lost when developing internal primers (Shan et al. 1999).

Properties of the mapped region

A genetic map (Lerceteau-Köhler et al. 2003) has suggested that the meiotic behaviour in strawberry is mixed, partly disomic and partly polysomic. The four AFLP and the two SCAR markers were mapped to the female group F4 of our reference map, a group comprising 17 markers linked in coupling phase. According to Sorrels (1992) and Wu et al. (1992), this association in the coupling phase suggests at least partial polysomic inheritance. Under such inheritance, repulsion-phase linkage is difficult to characterise (Sorrells 1992). Consequently, co-dominant markers in this type of linkage group would not be detectable using a classical program such as Mapmaker, as we observed for our dominant SCAR markers.

Polyploidisation in the cultivated strawberry is illustrated by the complex of bands obtained with the amplification of the SCAR STS_Rca2_417. In a preliminary study aimed at determining the association of homoeologous chromosomes using orthologous loci, two bands of the complex were located on two different homoeologous linkage groups in the reference mapping population 'Capitola'×'CF1116' (unpublished data).

Presence of the SCARs in 43 genotypes and cultivars

Results showed that 81.4% of the resistant/susceptible genotypes were correctly predicted using STS-Rca2_417. The absence of SCAR markers in some of the resistant cultivars may be explained by recombination between the resistance gene and its closest SCAR marker STS-Rca2_417, which could then be further inherited as observed for the genotype 'US292' and one of its parents 'Arking'. Recombination frequency, which depends on the distance between the resistance gene and the SCAR marker (0.6 cM for the 'Capitola'×'Pajaro' F_1-population) might vary between populations. In the 'Capitola' × 'CF1116' F_1-population, which is our reference population, we have estimated the distance to be 1.85 cM (unpublished results). Similar variation depending on genotypes or populations studied has been described previously in other species (Melotto et al. 1996; Ariyarathne et al. 1999). The absence of SCAR markers in resistant cultivars may also be due to the existence of other alleles or genes controlling resistance. This latter hypothesis is poorly supported by the narrow genetic basis of the cultivated strawberry (Sjulin and Dale 1987; Hancock and Luby 1995), which would rather suggest that very few origins of the resistance would be involved. However, resistance to *C. acutatum* was observed in *F. chiloensis* and *F. virginiana* (J.L. Maas, personal communication), the parental species of the *F.* × *ananassa* and in the diploid *F. vesca* (Denoyes-Rothan et al. 2005). Further work such as an allelic test or molecular analysis of a population using a resistant parent that does not possess the SCAR markers could differentiate between the two hypotheses.

Use of the markers in MAS

Since the SCAR markers we developed are present in the majority of the resistant genotypes (75% of the resistant genotypes presented the SCAR marker STS-Rca2_417), they are of particular interest for detection of resistance to *C. acutatum* pathogenicity group 2 in a program using MAS, provided that a pre evaluation of the presence of the markers in the parental materials was carried out. In the F_1-progeny of the 'Capitola' × 'Pajaro' cross, the recombination percentage of 2.8% between the dominant gene and the SCAR STS-Rca2_240 is below the value observed in escape seedlings for susceptible genotypes. We have found that on average 5% of the 83 susceptible F_1S_1-seedling families were escapes. Denoyes-Rothan et al. (2005) evaluated S_1-seedlings from eight susceptible cultivars and found a mean of 3% escapes. Therefore, the SCAR STS-Rca2_240 is a powerful tool for identifying resistant selections when their parents possess this SCAR marker. With a recombination percentage of 0.6% between *Rca2* and the SCAR STS-Rca2_417, this latter marker is particularly interesting in selection, especially if we succeed in the development of allele-specific primers.

Detection of resistance in a breeding program using MAS is of particular interest for anthracnose resistance. Since *C. acutatum* is subjected to statutory quarantine requirements in EEC (29/2000 Directive EC) all inoculations have to be performed in a controlled environment, which is not available in all breeding institutes. Furthermore, since both *C. acutatum* pathogenicity groups may be present in some fields, it is necessary to select for resistance to both groups. Due to competition between isolates of the two pathogenicity groups of *C. acutatum* (unpublished data), the selection scheme requires separate inoculations with isolates of each group. The use of the SCAR markers developed in this study in replacement of laborious biological tests for the monogenic resistance would allow selection independent of climatic conditions and without the use of isolates of *C. acutatum*. Moreover, the selection of resistant genotypes should speed up since the test may be carried out on young seedlings very early in the breeding program.

In order to develop resistance to both pathogenic groups by pyramiding the dominant resistance gene and the chromosomic regions involved in resistance to anthracnose, further work is required to detect quantitative trait loci (QTLs) linked to resistance to *C. acutatum*, pathogenicity group 1 (Denoyes and Baudry 1995). The combination of these different types of resistance would assure better control of the propagation of the disease and a reduction of the associated costs.

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