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Molecular marker-assisted introgression of the wild *Solanum commersonii* genome into the cultivated *S. tuberosum* gene pool

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Abstract A breeding scheme involving ploidy and EBN manipulations was set up to overcome the interspecific barriers existing between the cultivated *Solanum tuberosum* and the wild species *S. commersonii*. Three backcross generations were obtained that were analyzed by means of molecular markers in order to verify the occurrence of recombination between homeologous chromosomes, the extent of the wild genome carried in each backcross, and the efficiency of introgressing useful genes. Twenty *commersonii*-specific RFLPs provided evidence for recombination on 5 out of 12 chromosomes; for the others no more than 1 *commersonii*-specific marker was found. Thirty-four *commersonii*-specific RAPDs and 61 *commersonii*-specific AFLPs were used to estimate the wild genome content in the BC_1, BC_2, and BC_3 generations. The mean value of *commersonii*-specific markers was 93% and 91% in the BC_1, 74% and 76% in the BC_2, and 31% and 26% in the BC_3 using RAPDs or AFLPs, respectively. Efficiency of the breeding scheme was evaluated by monitoring across these progenies the introgression of *S. commersonii* resistance to tuber soft rot caused by *Erwinia carotovora*. Eleven and five resistant genotypes were found among BC_2 and BC_3 hybrids, respectively. The same progenies were also evaluated for chromosome number and tuber traits. For all analyzed traits except stolon length, all BC_2 and BC_3 hybrids resembled the *tuberosum* type. In order to choose the best genotypes to obtain the following backcrosses, we performed, in each generation, a negative molecular-assisted selection against the wild genome combined with selection for resistance to *Erwinia* spp. and other traits.

Keywords *Solanum commersonii* · Backcrosses · Assisted-negative-selection · Molecular markers · Resistance to *Erwinia* spp.

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Introduction

Exotic *Solanum* germplasm is a rich source of valuable genes capable of providing resistance to pathogens, improving the tolerance to abiotic stresses, and increasing yield and tuber quality traits. Up till now, wild potato species have played an important role as a resistance source for the breeding of the tetraploid (2n=4x=48) cultivated potato *Solanum tuberosum* (tbr), and many genes have already been incorporated into modern varieties (Ross 1986). However, there is still great potential in exploiting useful genes from the wild potatoes, and such genes are generally available for breeding, since most wild and cultivated potato species can hybridize due to the poor differentiation among chromosomes of different species (Peloquin et al. 1989a). Peloquin et al. (1989b) proposed a breeding approach whereby haploids (2n=2x=24) extracted from cultivated varieties can be crossed with 24-chromosome species to capture genes of interest from the wild species. Newly developed diploid hybrids can be selected for the target trait(s) and for 2n gamete production. Through the function of 2n gametes, traits of interest can then be transferred to the tetraploid level simply by employing unilateral sexual polyploidization (4x-2x crosses).

Unfortunately, in some cases this scheme fails due to strong isolating mechanisms, the most common of which is the failure of endosperm development (Hermsen 1994). As a means of explaining the crossing behavior of different species, each species has been assigned an Endosperm Balance Number (EBN) (Johnston et al. 1980). It was hypothesized that crosses are successful when a 2:1 maternal to parental EBN ratio in the hybrid

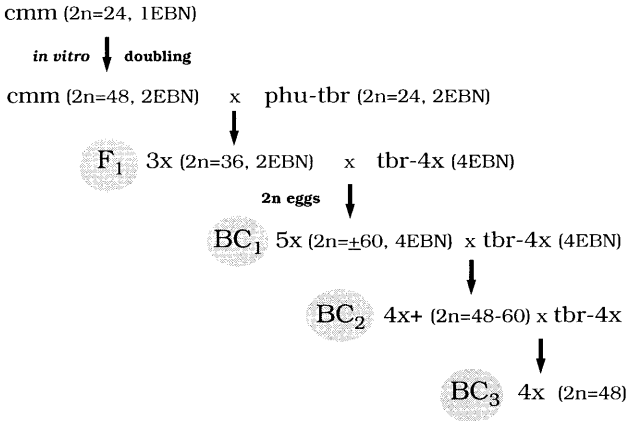

Fig. 1 Backcross breeding scheme adopted to introgress the wild species *Solanum commersonii* into the *S. tuberosum* gene pool (for details see Materials and methods)

endosperm is present. This interspecific post-zygotic barrier can be circumvented through conventional breeding, based on EBN and ploidy manipulations aimed at equalizing the parental EBNs, or through biotechnological approaches such as somatic hybridization and embryo rescue (Hermsen 1994). Among the 1EBN diploid species, *Solanum commersonii* (cmm) is a source of several valuable traits, such as resistance to pathogens (i.e., *Erwinia* spp.), high dry matter content of tubers, and resistance to low temperatures, including both freezing tolerance and the ability to cold acclimate (Hanneman and Bamberg 1986; Palta 1994). Due to the high value of this wild species as a useful germplasm source, different approaches have been used by several authors to overcome its sexual isolation (Novy and Hanneman 1991; Masuelli and Camadro 1992; Cardi et al. 1993).

Recently, we developed a backcross breeding scheme (Fig. 1) using triploid F_1 (2n=3x=36) cmm-*S. phureja*-tbr hybrids as female parents in 3x×4x crosses with tetraploid tbr. Through the function of 2n eggs, these crosses successfully produced the BC_1 progeny, which was male and female fertile in crosses with 4x tbr varieties, thus allowing the production of the BC_2 generation (Carputo et al. 1997a).

However, the flow of useful genes from the wild cmm to the cultivated tbr is not guaranteed by the circumvention of these barriers, since the introgression of genes from one to the other species requires that both pairing and recombination between homeologous chromosomes occur in the obtained hybrids. Pairing between cmm and tbr chromosomes has already been proven both in the triploid F_1 and pentaploid BC_1 hybrids (Barone et al. 1999; Carputo et al. 1999), but it has not yet been ascertained whether pairing is actually followed by crossing-over and recombination. The use of species-specific molecular markers would greatly facilitate the study of recombination among homeologous genomes, as already proposed for different *Solanum* species (McGrath et al. 1994; Masuelli et al. 1995).

In the investigation reported here breeding assisted by molecular markers was applied with two objectives: (1) to verify the occurrence of recombination between cmm and tbr genomes, and (2) to monitor the degree of wild cmm genome still present in hybrids of each backcross, in order to perform assisted negative selection. In addition, in order to verify the effectiveness of our backcross scheme in transferring genes from cmm to tbr we chose to follow the introgression of the resistance to tuber soft rot by *Erwinia carotovora*.

Materials and methods

Plant material

Triploid hybrids (2n=3x=36) between an *in vitro* doubled accession of *S. commersonii* (2n=4x=48) and *S. phureja*×*S. tuberosum* hybrids (2n=2x=24) were obtained as reported by Carputo et al. (1995). They were crossed as females with 4x tbr (3x×4x) to obtain BC_1 genotypes. One BC_1 pentaploid (P5, 2n=5x=60) was used in 5x×4x and 4x×5x crosses with 4x varieties to produce the BC_2 progeny (Carputo et al. 1997a). BC_2 genotype PTHF17 (2n=4x=48) was backcrossed with tbr to obtain the BC_3 progeny. Four F_1, 11 BC_1, 43 BC_2 and 20 BC_3 progeny were used for molecular analyses and resistance tests.

All pollinations were carried out in the greenhouse using emasculated flowers. The resulting seeds were treated with gibberellic acid (1500 ppm) for 24 h to break dormancy and sown in styrofoam trays, the seedlings obtained were transplanted to pots in a temperature-controlled greenhouse to collect root tips for mitotic analysis and leaves for molecular analysis. Tubers of cmm (PI 243503) and of 4 tbr tetraploids (advanced selection WIS 482, cvs. Blondy, Carmine, Tollocan) were planted in the same greenhouse to collect leaves. At harvest, tuber characteristics were recorded.

Cytological analysis

The chromosome number of the genotypes used was determined by counting the chromosomes in the root-tip cells. Root tips were treated with 0.29 g/l 8-hydroxyquinoline for 5 h and subsequently fixed in a 3:1 ethanol to acetic acid solution for 48 h. Following hydrolysis for 55 min in 5 *N* HCl at room temperature, the root tips were stained for at least 2 hours with Schiff's reagent and squashed in 45% acetic acid.

DNA extraction

Total genomic DNA was extracted only from genotypes that were highly vigorous, thus allowing enough leaves to be collected without limiting plant growth. For RFLP analysis the total genomic DNA was extracted following the CTAB protocol with minor modifications (Doyle and Doyle 1990). For RAPD and AFLP analyses the total genomic DNA was obtained using the extraction kit DNeasy Plant Mini Kit from Qiagen.

Restriction fragment length polymorphism (RFLP) analysis

Five micrograms of total DNA from each parental genotype was digested with three restriction enzymes (*Eco*RI, *Eco*RV and *Hind*III, 4–5 U/µg DNA) at 37°C for 16 h. DNA fragments were separated by electrophoresis on 0.8% agarose gels and transferred by capillarity onto charged nylon membrane (Hybond N+, Amersham) as described by Sambrook et al. (1989). Labelling of DNA clones with α-[^{32}P]-dCTP, prehybridization, hybridization and washes of membranes were as described by Gebhardt et al. (1989). Membranes were exposed to X-ray film with two intensifying screens at –80°C for 3 days. In order to select cmm-specific

RFLPs, we used 50 DNA clones which have a known position on the potato RFLP map (Gebhardt et al. 1991) as probes: 30 derived from a cDNA (CP) or a genomic DNA (GP) library of potato (Gebhardt et al. 1991) and 20 from a genomic library of tomato (TG) (Tanksley et al. 1992).

Random amplified polymorphic DNA (RAPD) analysis

DNA from each genotype was amplified by using the polymerase chain reaction (PCR) conditions described by Williams et al. (1990). Each reaction consisted of 1× buffer (50 m*M* KCl, 10 m*M* TRIS-HCl, pH 8.3, 1.5 m*M* $MgCl_2$, 0.001% gelatin), 0.1 m*M* of each dNTP, 0.2 µ*M* primer, 1.0 U *Taq* DNA polymerase and 20 ng genomic DNA. Amplification was performed in a Perkin-Elmer/Cetus DNA thermal cycler programmed for 45 cycles of 1 min at 94°C, 1 min at 35°C, 2 min at 72°C, followed by 7 min at 72°C. Amplification products were separated by electrophoresis on 1.5% agarose gel in 1×TAE buffer. In order to select cmm-specific RAPDs, a total of 155 10-mer oligonucleotide primers were examined: 40 were commercial 10-mers (Operon Technologies, Alameda, Calif.) from kits AN and H, 100 were commercial 10-mers from the University of British Columbia (UBC set 100/1), and 15 were 10-mers randomly designed (Sebastiano et al. 1999) and commercially synthesized by M-Medical (Italy). To verify the cmm-specificity of the selected RAPDs, we blotted the PCR gels of parental genotypes onto a nylon membrane (Amersham, N^+) and then hybridized these with the cmm-specific RAPD fragments excised from the gel and labelled with α-[^{32}P]-dCTP.

Amplified fragment length polymorphism (AFLP) analysis

Selection of cmm-specific AFLPs was performed on parental genotypes using the method described by Vos et al. (1995) and the commercially available AFLP kit and protocol (Gibco-BRL AFLP analysis System I, Life Technologies, Gaithersburg, Md.) which employs *Eco*RI and *Mse*I as restriction enzymes. For selective amplification ten combinations of primer were used (*Eco*RI+ACT and *Mse*I+CAT, *Eco*RI+ACT and *Mse*I+CAG, *Eco*RI+ACT and *Mse*I+CAC; *Eco*RI+AGC and *Mse*I+CAA, *Eco*RI+AGC and *Mse*I+CTA; *Eco*RI+AGG and *Mse*I+CAG, *Eco*RI+AGG/ and *Mse*I+CAA, *Eco*RI+AGG and *Mse*I+CTT; *Eco*RI+AAC and *Mse*I+CAG; *Eco*RI+ACC and *Mse*I+CAA), and the fragments obtained were resolved on a 6% denaturing polyacrylamide gel run at a constant power of 50 W for 3 h. Gels were transferred to Whatmann 3 MM paper and dried for 2 h at 80°C on a gel dryer (BioRad). They were then exposed to X-ray films for 24 h.

Assessment of recombination events

The RFLP analysis was used to assess recombination events between cmm and tbr chromosomes. A recombination event was assumed when 1 of at least 2 cmm-specific markers mapping on the same chromosome was absent in the progeny. In order to get informative markers for this purpose, we first based our selection of cmm-specific RFLPs on the screening of RFLP markers, which are truly chromosome-specific and, therefore, detected only one locus on reported potato RFLP maps (Gebhardt et al. 1989, 1991, 1994). In addition, informative cmm-specific RFLPs should be homozygous in the parental cmm genotype, and this condition was verified through the segregation analysis of these markers (this laboratory, unpublished results) on a selfed progeny coming from a tetraploid *Solanum tuberosum* (+) *S. commersonii* somatic hybrid (Cardi et al. 1993).

Resistance to *Erwinia carotovora*

The *Erwinia* strain used was Ecc 009 obtained from the International Potato Center. To inoculate tubers, we obtained bacterial suspensions of 1×10^7 colony-forming units/ml from cultures maintained on nutrient agar at 25°C for 48 h. All the BC_1, BC_2, and BC_3 hybrids, their parents and control varieties Spunta and Desiree were screened for resistance to tuber soft rot. The screening technique reported by Austin et al. (1988) was used. After sterilization with 0.5% sodium chloride for 20 min, 3–5 holes (2 mm wide and 20 mm deep) were drilled in each tuber. One hole was inoculated with sterile water as a control, and all the others were inoculated with 20 µl of bacterial suspension. Seven to ten tubers of each clone were inoculated with each strain. After a 72-h incubation at 24°C in a dew chamber, the tubers were cut vertically through the injection points, and the diameter of decay was measured. Based on the width of the rotted area, an arbitrary scale was used to classify clones as resistant (diameter of rotted area: 2–4 mm), intermediate (4–6 mm), susceptible (>6 mm) (Carputo et al. 1997b).

Results

Evidence for recombination

The RFLP analysis based on the use of markers with a known location on the potato RFLP map (Gebhardt et al. 1991) provided evidence of recombination between tbr and cmm chromosomes. In particular, out of 50 probes analyzed on parental cmm and tbr genotypes, 20 (40%) were considered cmm-specific RFLPs since they identified fragments which were only present in the cmm parent and absent in the four tbr genotypes used in the backcross scheme (Fig. 2). These cmm-specific RFLPs were then screened on the triploid F_1 genotypes, and they were always present. In order to verify if recombination occurred between cmm and tbr genomes, we tested for the presence of cmm-specific RFLPs on the BC_2 progeny, since the available BC_1 genotypes were few (11) and came from different 3x×4x cross combinations. All the cmm-specific RFLPs, except for the 3 mapping on chromosome XI (GP38-GP125-TG26), were present in the BC_1 genotype P5 that was used to obtain the BC_2 progeny. This provided evidence that this cmm chromosome (XI) was lost due to disrupted meiotic pairing during the megagametogenesis of the F_1 triploid parent.

The cmm-specific RFLPs which map on the 11 other chromosomes of the potato RFLP map were tested on selected 30 BC_2 genotypes coming from the cross P5× Blondy (Fig. 2). By scoring the presence or absence of these RFLPs on the BC_2 genotypes it was possible to highlight some recombination events between cmm and

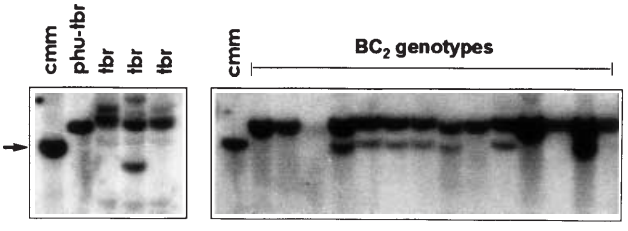

Fig. 2 RFLP analysis with the potato probe GP76. **Left** Selection of 1 cmm-specific RFLP (*arrow*) on parental genotypes, **right** screening of the probe GP76 on 14 genotypes from the BC_2 progeny P5× Blondy

Table 1 RFLP analysis of 30 BC_2 genotypes: number of analyzed RFLPs on parental genotypes, number and code of cmm-specific RFLPs, evidence and number of recombinants for each chromosome

Chromosome	Analyzed RFLPs (no.)	cmm-specific RFLPs		Recombination event	Recombinant genotype[a] (no.)
		Number	Code		
I	3	1	CP46	–	–
II	3	2	GP26-TG31	+	7
III	3	1	GP25	–	–
IV	6	2	GP75-TG123	+	13
V	5	1	GP22	–	–
VI	5	1	TG118	–	–
VII	4	3	GP27-GP77-TG61	+	5, 9
VIII	4	2	GP92-GP126	+	8
IX	3	2	GP39-TG35	+	6
X	3	1	TG63	–	–
XI[b]	–	–	–	–	–
XII	3	1	TG28	–	–

[a] The two values reported in this column for chromosome VII refer to the number of recombinants between markers GP27 and GP77, and between markers GP77 and TG61, respectively
[b] Data on chromosome XI are not reported since the three cmm-specific RFLPs mapping on this chromosome were not present in the BC_1 genotype P5, which was the female parent of the BC_2 progeny

tbr chromosomes. Table 1 reports the RFLPs tested for each chromosome, the ones that were cmm-specific and evidence of recombination. At least 3 markers per chromosome were tested. In some cases (chromosomes II and IX) they were enough to find 2 cmm-specific markers, which is the minimum number required to provide evidence of recombination. In other cases (chromosomes I, III, X and XII) only cmm-specific marker was found, even for chromosomes V and VI for which 5 markers were tested. Out of 11 chromosomes analyzed, 5 had 2 or 3 cmm-specific RFLPs: for all of them recombination events were noted. In fact, when the cmm-specific RFLPs for each chromosome were screened, in some BC_2 genotypes one RFLP was present but the other was absent; this event is only possible if crossing over and recombination occur between the 2 markers. The frequency of recombination events was very variable, this also depends on the reciprocal position of these RFLPs and their position with respect to the centromere (data not shown). A larger progeny will have to be analyzed to better define the percentage of recombination events, since 30 genotypes were not enough to clearly establish the recombination frequencies but only allowed the occurrence of recombination to be ascertained.

Degree of introgression

Both RAPDs and AFLPs were used to estimate the percentage of the wild genome still present in the different backcross generations. Out of 155 primers tested on parental genotypes, 41 (26.4%) identified cmm-specific RAPDs since they amplified fragments only in cmm but not in the tbr parental genotypes. When these cmm-specific fragments were used as probes on PCR gels, only 34 of them (21.9%) clearly demonstrated they were highly cmm-specific; these were therefore used for monitoring introgression. All of the markers except for 1 (UBC12) were present in the F_1 triploids, and this marker

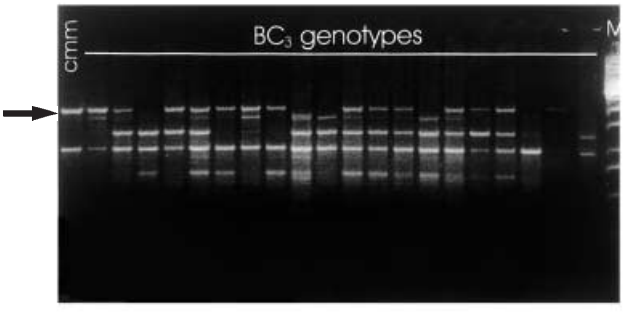

Fig. 3 RAPD analysis with primer OPAN 1: segregation of 1 cmm-specific RAPD (*arrow*) on 20 BC_3 genotypes

was eliminated from further analyses, since it identified one heterozygous locus. Out of the ten primer/enzyme combinations used on the parental genotypes, 61 cmm-specific AFLPs were identified, with a mean of 6.1 per primer combination, a minimum of 3, and a maximum of 11. These RAPD and AFLP markers were analyzed on the backcross generations: 11 BC_1, 43 BC_2 and 20 BC_3 backcross genotypes (Figs. 3, 4). Most BC_1 genotypes (9 out of 11) showed an index of 90%–100% cmm-specific RAPDs; 46.9% of BC_2 genotypes had a value of cmm-specific RAPDs ranging from 60% to 70%, whereas in the BC_3 progeny 47.4% of the genotypes had a value falling in the 20–30% class. As regards the percentage of cmm-specific AFLPs, 50% of BC_2 genotypes showed a value of 70–80% and 37% of BC_3 genotypes a value of 20–30%. Figure 5 shows the frequency distribution of plants in different introgression classes based on RAPD and AFLP analyses. Table 2 reports the mean values of both cmm-specific RAPDs and AFLPs in the different backcross generations. With respect to the RAPD analysis, the mean was 99.0% in the F_1, 93.1% in BC_1, 73.9% in BC_2, and a marked reduction to 31.2% in BC_3. These values were mainly confirmed through AFLP analysis that showed means of 91.6%, 75.9%, and 26.2% in the BC_1, BC_2 and BC_3 generations, respectively.

Table 2 Estimation of the wild cmm genome present into cmm-tbr F_1, BC_1, BC_2, and BC_3 hybrids through the percentage of cmm-specific RAPDs and AFLPs (mean and range)

Generation	Cross	Analyzed plants (no.)	Percentage of cmm-specific RAPDs[a]		Percentage of cmm-specific AFLPs[a]	
			Mean	Range	Mean	Range
F_1	cmm×tbr-phu	4	99.0	96–100	100.0	–
BC_1	3x×W482	11	93.1	80–100	91.6	82–98
BC_2	P5×Blondy	43	73.9	48–97	75.9	55–91
BC_3	PTHF17×Blondy	20	31.2	19–42	26.2	11–40

[a] Number of cmm-specific markers observed in each genotype/no. of cmm-specific markers analyzed) ×100

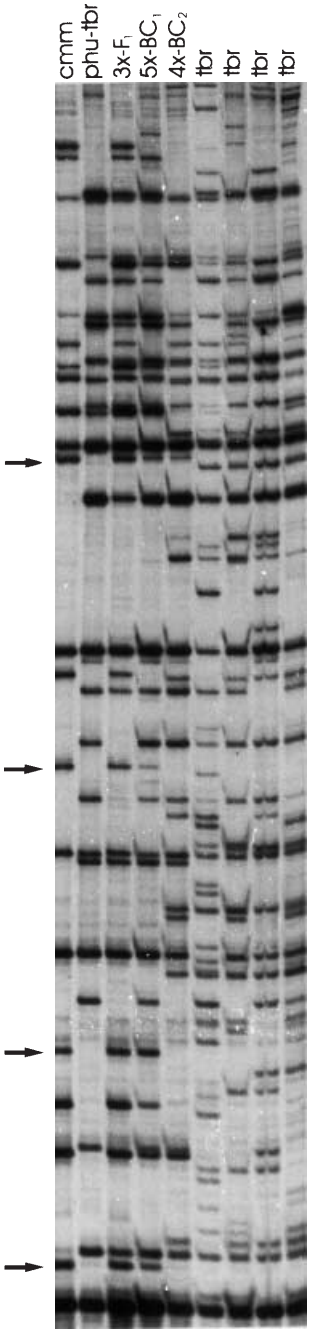

Fig. 4 AFLP analysis with the primer combination *Eco*RI+ACT and *Mse*I+CAC. Selection on parental genotypes of 4 cmm-specific AFLPs (*arrow*)

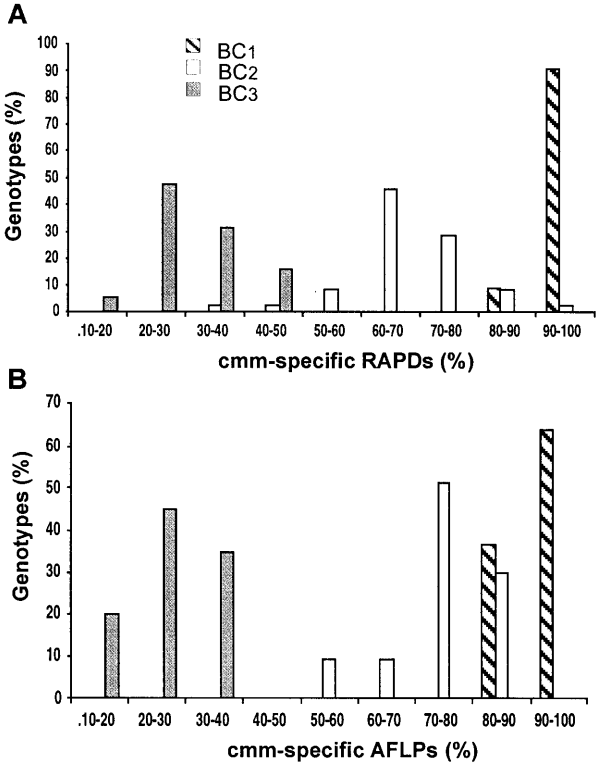

Fig. 5 Frequency distribution of BC_1, BC_2, and BC_3 genotypes in different introgression classes on the basis of RAPD (**A**) and AFLP (**B**) analysis

Selection of hybrids

In addition to the percentage of cmm-specific markers, also used as tools to select the best genotypes to backcross to tbr were (1) chromosome number, (2) resistance to *Erwinia carotovora*, and (3) tuber traits.

The chromosome number has already been reported for the F_1 (2n=3x=36) (Carputo et al. 1995) and BC_1 (2n=58–67) (Barone et al. 1999). As for the BC_2 progeny, the chromosome number of 43 BC_2 genotypes varied from 48 to 57, most genotypes having between 51 and 54 chromosomes. BC_3 genotypes from 4x×4x crosses are expected to be tetraploids. A random sample of genotypes confirmed this assumption.

As for resistance to tuber soft rot, the parental cmm accession was highly resistant (Sirianni 1997), as well as

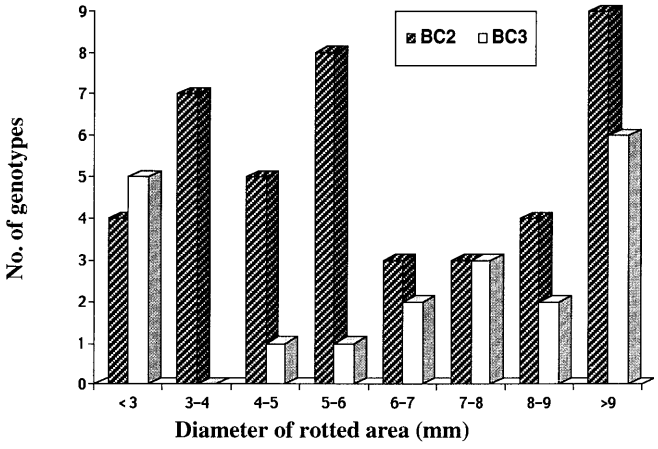

Fig. 6 Distribution of BC_2 and BC_3 genotypes for the resistance to *Erwinia carotovora* (resistant genotypes showed a diameter of rotted area <4 mm)

the triploid F_1 hybrids (data not shown). Out of 11 BC_1 genotypes tested, 2 were highly resistant (genotypes P5 and P3), while the others were moderately resistant or susceptible. As positive control the parental tbr varieties Blondy and Tollocan were tested; these were highly susceptible with a diameter of rotted area >9 mm. The BC_1 genotype P5 was chosen to obtain the BC_2 progeny. Among these BC_2 genotypes, segregation for resistance to *Erwinia* spp. was observed (Fig. 6) with 11 genotypes being classified as resistant (diameter of the rotted area <4 mm) and 13 as moderately resistant (diameter of the rotted area 4–6 mm). Within the resistant and moderately resistant genotypes, PTHF17 was chosen as the parent for obtaining the BC_3 progeny in that it associated a good resistance (diameter of rotted area between 3–4 mm in replicated tests) with the lowest values of cmm-specific RAPDs (48%) and AFLPs (55%). Interestingly, based on the RFLP analysis performed on the BC_2 progeny, cmm-specific markers were lacking on chromosomes VII and X, as well as on chromosome XI, in genotype PTHF17 (see Table 1). This suggests that genes conferring resistance to *Erwinia carotovora* are not localized on these chromosomes. Among 20 BC_3 genotypes five highly resistant and two moderately resistant were classified (Fig. 6). Among these 5 highly resistant genotypes the value of the cmm-specific markers ranged from 23% to 30%.

As for morphological evaluation, while the F_1 triploid hybrids clearly resembled the cmm parent, in the BC_1, BC_2, and BC_3 generations the plants had morphological aspects more similar to the tbr type in terms of skin colour, flesh colour, and eye depth. The only trait that mostly resembled the cmm type in both the BC_2 and BC_3 progenies was stolon length. As for tuber yield, good tuber production as well as good tuber size and shape were observed in the BC_2 and BC_3 progeny. Twenty-seven per cent of the BC_2 genotypes and 16% of the BC_3 produced more than 2.0 kg/plant, which is clearly superior to that of tbr varieties (1.4 kg/plant and 1.5 kg/plant for Tollocan and Blondy, respectively).

Finally, considering the resistance to *Erwinia* spp., morphological and tuber traits together with the wild genome content, out of 20 BC_3 genotypes 2 which combined all the best traits were chosen to make crosses with different tbr varieties with the aim of obtaining BC_4 progenies.

Discussion

The high value of the backcross scheme used to introgress the genome of *S. commersonii* into the cultivated *S. tuberosum* relies on the demonstration that EBN barriers to interspecific hybridization between the 1EBN cmm and the 2EBN tbr species have been overcome through direct *in vivo* ploidy manipulation. Phylogenetic studies in the genus *Solanum* (Debener 1990; Matsubayashi 1991) demonstrated that *S. commersonii* and *S. tuberosum* are closely related. This close affinity made it possible to enhance genetic recombination, as we observed on the 5 chromosomes for which more than 1 cmm-specific RFLP was detected. This led to the conclusion that, at least for these chromosomes, it would be possible to achieve stable gene introgression between cmm and tbr. On the contrary, the same close affinity could explain our difficulty in finding informative cmm-specific RFLPs (only 20 were found) for the other 7 chromosomes. In fact, when studying more distantly related species, such as *S. brevidens* and *S. tuberosum*, various authors found a higher percentage of species-specific RFLPs, which highlighted recombination events for all the 12 chromosomes (Williams 1993; McGrath 1994).

The occurrence of homeologous recombination provided evidence that the introgression of wild species traits is possible between such sexually isolated incompatible species. However, the transfer of target genes into the recipient genome must take place with the concurrent exclusion of genes responsible for undesirable wild traits through subsequent backcrosses with the cultivated species, which is a kind of negative selection. Compared to conventional backcrosses, the one performed here to introgress cmm into the tbr genetic background is peculiar, since it involves crosses between individuals at different ploidy levels and because is based on 2n egg production, whose formation mechanisms can greatly influence the chromosome complement of the hybrid progeny. Therefore, one objective of this work was to monitor the degree of wild cmm genome still present in the hybrids of each generation which, due to the unique chromosome constitution of these genotypes (F_1 triploids, BC_1 pentaploids, BC_2 tetraploid-pentaploids), can clearly differ from that predicted for a usual backcross scheme. Two different molecular markers used for this purpose, RAPDs and AFLPs, mainly showed the same results in terms of percentage of the wild genome present in the BC_1, BC_2 and BC_3 generations. Therefore, our analysis, which aims to evaluate the wild genome content in the further backcross generations will continue

only with the use of AFLPs, which were confirmed to be the most suitable markers for potato genome analysis (Milbourne et al. 1997). The low wild genome reduction observed from F_1 to BC_1 is due to the presence of two cmm versus one tbr genome in the F_1 triploids. A higher reduction was observed from BC_1 to BC_2, even though in the pentaploid genome of the parental BC_1 genotype two cmm genomes are still present as a consequence of the production of eggs with an unreduced chromosome number in the parental triploid. This reduction could be explained by the occurrence of homeologous pairing between cmm and tbr chromosomes and, thus, to both recombination and segregation. The severe reduction from BC_2 to BC_3 progenies may be due to the presence, for the first time, of only one copy of most cmm chromosomes.

The progressive loss of the wild genome combined with recombination between the cmm and tbr genomes made it possible to achieve the practical purpose of this breeding scheme – that is to transfer useful genes from the wild species *S. commersonii* into the cultivated gene pool through backcrosses. In order to verify the effectiveness of this breeding scheme, we chose to follow the introgression of resistance to *Erwinia* spp. In each generation, resistant genotypes with many morphological traits resembling the cultivated parent were selected. This is particularly important given that resistant varieties are not available and that *Erwinia* control is often ineffective (Zimnoch-Guzowska and Lojkowska 1993). In addition, the negative molecular-assisted analysis performed to select superior genotypes showing a low wild genomic content associated with resistance to *Erwinia* spp. could be useful to describe a kind of graphical genotype of the selected hybrids, based on the cmm-specific markers scored in different backcross generations. This could probably lead to the identification of markers linked to the target resistant gene(s), which would be very useful for screening other populations.

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