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Selection for aneuploid potato hybrids combining a low wild genome content and resistance traits from *Solanum commersonii*

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Abstract A breeding scheme based on the production of progenies with odd ploidy was followed to introduce useful genes from the wild *Solanum commersonii* (cmm) into *S. tuberosum* (tbr) genome. Hybrids from $5x \times 4x$ crosses were characterized for traits of interest, and selection was assisted by amplified fragment length polymorphism (AFLP) analysis. As expected, most of the hybrids were aneuploids, with a trend towards a low degree of aneuploidy. Despite the fact that aneuploidy has often been associated with a reduction in male and female fertility, most of the hybrids were fertile following crosses with tbr, making it possible to produce viable offspring. A screening for resistance traits deriving from cmm was also carried out. With respect to freezing resistance, the killing temperatures of cold-acclimated genotypes were distributed between the wild and cultivated parental values, with some hybrids displaying an acclimation capacity higher than 3°C. A wide variability was also found for tuber soft rot resistance, and hybrids with high levels of resistance were identified. Selection of hybrids was based on a two-stage scheme that consisted of conventional phenotypic selection followed by an estimation of the wild genome content still present in order to identify hybrids combining noteworthy traits with a low wild genome content. Previously selected cmm-specific AFLPs were used to monitor the degree of wild genome content still present in each hybrid. The percentage of cmm-specific markers

ranged from 59% to 91%, with an average value of 75%. AFLP analysis was employed to assist in the selection of valuable hybrids for further breeding efforts.

Introduction

Wild tuber-bearing *Solanum* species represent an important source of the allelic diversity and genes necessary to improve the narrow genetic base of the cultivated potato, *Solanum tuberosum* ($2n=4x=48$) (tbr). Most are diploid ($2n=2x=24$), and a simple and efficient approach for their use involves the production of tbr haploids ($2n=2x=24$) prior to crossing with diploid species. The return to the tetraploid level of the cultivated potato may be achieved through sexual polyploidization, which allows the production of heterotic tetraploids (Peloquin et al. 1999). Exploitation of some diploid *Solanum* spp. through this relatively simple approach is sometimes hampered by post-zygotic barriers which cause the degeneration of the haploid-wild species hybrid endosperm and, consequently, embryo abortion (Johnston and Hanneman 1982). Diploid incongruent species (amongst others *S. brevidens*, *S. bulbocastanum*, *S. commersonii*, *S. etuberosum*, *S. jamesii*) are particularly interesting for their resistance to biotic and abiotic stresses, and breeders have attempted either somatic fusion or embryo rescue for their exploitation (Singsit and Hanneman 1991; Cardi et al. 1993; Watanabe et al. 1995; Helgeson et al. 1998; Gavrilenko et al. 2003). A simple, flexible way to overcome post-zygotic barriers is based on bridge crosses. This approach is particularly suitable in the potato, where whole genomes can be easily manipulated through the functioning of $2n$ gametes. Following a bridge strategy, Carputo et al. (1997a) produced sexual hybrids between *S. tuberosum* and incongruent $2x$ (1EBN, endosperm balance number) *S. commersonii* (cmm). Cmm is of great interest due to its resistance to low temperatures, bacterial wilt, tuber soft rot and a high specific gravity of tubers. The breeding strategy carried out was based on doubling the chromosome number and EBN value of the $2x$ (1EBN) cmm followed

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by crosses between the 4x(2EBN) cmm clone that developed and a *S. tuberosum*-*S. phureja* hybrid. Through the functioning of 2n ovules, the triploid (2n=3x=36) F₁ progeny obtained was successful backcrossed to tetraploid varieties to generate BC₁ pentaploid (2n=5x=60) genotypes. Further 5x × 4x crosses with tbr were made with relative ease. One peculiar aspect of the breeding scheme used is related to the fact that pentaploids are known to produce gametes with various chromosome numbers (potentially from 24 to 36). Thus, the production of aneuploids is expected from 5x × 4x crosses.

In the literature on potato, scant information is available on aneuploids from pentaploid genotypes. The objective of the study reported here was to characterize a 5x × 4x progeny for cytological, fertility and resistance traits in order to select superior hybrids for further breeding efforts. To speed up the selection program and to identify genotypes combining noteworthy traits with a low wild genome content, we used AFLP markers for assisted selection.

Materials and methods

Plant material

Plant material consisted of 51 hybrids (hereafter coded PTH) generated from 5x × 4x crosses between the pentaploid sexual cmm-tbr hybrid MCPH 1 and the tbr variety *Blondy* (Carputo et al. 1997a). The parental genotypes MCPH 1 and *Blondy*, tbr cultivars (*Désirée*, *Spunta* and *Tollocan*) and wild cmm were also included as controls and/or employed in crosses with PTH. Root tips of in vitro-grown plants were used for mitotic analysis before these were transplanted into pots in a temperature-controlled greenhouse to produce tubers for field evaluations.

Cytological analysis

To assess chromosome number, we carried out a mitotic analysis according to Carputo (2003). Hybrid distribution in ploidy-level classes was compared to expected binomial distribution (0.5+0.5)¹² assuming random transmission of the extra chromosome from the 5x parent. The statistical significance of differences between observed versus expected distribution was computed by χ^2 analysis.

Fertility analysis

Out of 51 genotypes planted, 46 flowered and were used for fertility analysis under screenhouse conditions in Camigliatello Silano (1,300 m a.s.l.). Male and female fertility of the PTH was analysed in reciprocal crosses with 4x tbr cultivars. Pollinations were carried out using emasculated flowers and freshly collected pollen. Seeds were extracted from ripened fruits, and fruit set and number of seeds per fruit were recorded. Seed germinability was determined for 23 PTH × tbr and 16 tbr × PTH cross combinations. During the flowering period, pollen stainability was determined by acetocarmine staining by counting at least 200 pollen grains. Hybrids with less than 5% stainable pollen were considered to be male-sterile. Differences in seed production between genotypes within each cross direction were computed by analysis of variance.

Screening for tuber soft rot resistance and specific gravity of tubers

One month after harvest, tubers from 48 PTH hybrids, parental genotypes (MCPH 1 and *Blondy*) and two tbr varieties (*Désirée* and *Spunta*) were screened. The diploid parents cmm and UP88-P5 were not used in that due to the small size of tubers produced the technique utilized to detect their resistance was different from that used for tubers of regular size and, consequently, the scale of resistance cannot be compared. Strain 009 of *Erwinia carotovora* subsp. *carotovora* (Ecc) was provided by the International Potato Centre, Lima, Peru. Five tubers per genotype were surface-sterilized and inoculated according to the procedure described by Carputo et al. (1997b). Following an arbitrary scale, genotypes with an average diameter of rotted area smaller than 4 mm, between 4 cm and 6 mm, and larger than 6 mm were classified as resistant, intermediate and susceptible, respectively. Differences between genotypes in the diameter of the tuber rotted area were indicated by Duncan's multiple range test.

The specific gravity of the tubers was determined at harvest using the weight in air-weight in water method.

Screening for freezing tolerance

A sample of PTH hybrids was evaluated for freezing tolerance under non-acclimated and acclimated conditions. The parental genotypes (MCPH 1 and *Blondy*), cmm and *Spunta* were also included as controls. The tests were carried out using the electrolyte leakage procedure described by Carputo et al. (2000). In brief, for the non-acclimated studies, four clonally propagated plants from each genotype were grown in a growth chamber at 18–20°C under a 16/8-h (light/dark) photoperiod with light supplied by cool-white fluorescent lamps (350–400 $\mu\text{mol m}^{-2} \text{s}^{-1}$). For the cold acclimation studies, two plants per genotype were transferred for 2 weeks to a cold room (2–4°C) under a 16/8-h (light/dark) photoperiod with light supplied at an intensity of 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Mature expanded leaves were put in culture tubes and submerged in a glycol bath at 0°C. Three replicates per genotype were used for each temperature treatment. The temperature was lowered 0.5°C every 30 min. The control treatment consisted of three replicates per genotype kept on ice at 0°C. After 30 min at the desired freezing temperature, the tubes were placed on ice to thaw overnight. The freezing tolerance (non-acclimated, NAFT; acclimated, AFT) for each genotype was determined by calculating the temperature at 50% freezing injury (LT₅₀) according to the logistic model described by Janáček and Prášil (1991). Statistical differences between the LT₅₀s of the genotypes tested were calculated with the programme LT50 ver. 2.1 (Janáček and Prášil 1991).

To monitor the inheritance pattern of freezing tolerance from cmm into PTH, degree of dominance (*d/a*) was calculated according to the formula (Falconer 1989): $d/a = (\text{Progeny mean} - \text{mid-parent value}) / (\text{mid-parent value} - \text{low-parent value})$. *d/a* is a numerical value to evaluate the tendency for the mean killing temperatures of PTH to deviate from the mid-parent value. The greater the phenotypic dominance, the greater the deviation of *d/a* from zero, with negative and positive values indicating dominance of the resistant (cmm) and susceptible (tbr) parent, respectively.

Amplified fragment length polymorphism analysis

AFLP analysis was carried out with 26 selected hybrids 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 *EcoRI* and *MseI* as restriction enzymes. Thirty cmm-specific markers, detected by seven previously selected combinations of primer (E-ACT/M-CAC; E-ACT/M-CAG; E-AGG/M-CAG; E-AGC/M-CAA; E-AGC/M-CTA; E-AGG/M-CAA; E-AGG/M-CTT), were used to evaluate the degree of wild genome still present

in the hybrids (Barone et al. 2001). Following selective amplification, the obtained fragments were separated by electrophoresis on 6% denaturing polyacrylamide gels and visualized by exposing X-ray films to the dried gel for at least 24 h. The presence/absence of cmm-specific AFLPs was scored for each hybrid/each primer combination, and the percentage of cmm-specific markers was calculated as follows: percentage cmm-specific AFLPs=(no. of cmm-specific markers observed in each PTH/ total no. of cmm-specific markers analysed)×100.

Data analysis

To identify hybrids with a desirable combination of traits, we calculated an evaluation index (EI) by assigning to each trait the following arbitrary scale: (1) stolon length, from 1 = very long to 4 = very short; (2) eye depth, from 1 = very deep to 4 = very shallow; (3) specific gravity of tubers, 1 = ≤ 1.070 , 2 = 1.080–1.070, 3 = 1.090–1.080, 4 ≥ 1.090 ; (4) resistance to *Erwinia* (Ecc), 1 = rotted area ≥ 8 mm, 2 = rotted area of 6–8 mm, 3 = rotted area of 4–6 mm, 4 = rotted area ≤ 4 mm; (5) non-acclimated freezing tolerance (NAFT), 1 = \leq killing temperature ($^{\circ}\text{C}$) of *Blondy*, 2 = killing temperature ($^{\circ}\text{C}$) of *Blondy*/–3 $^{\circ}\text{C}$, 3 = –3 $^{\circ}\text{C}$ /–4 $^{\circ}\text{C}$, 4 = \geq –4 $^{\circ}\text{C}$; (6) acclimated freezing tolerance (AFT), 1 = \leq killing temperature ($^{\circ}\text{C}$) of *Blondy*, 2 = killing temperature ($^{\circ}\text{C}$) of *Blondy*/–4 $^{\circ}\text{C}$, 3 = –4 $^{\circ}\text{C}$ /–5 $^{\circ}\text{C}$, 4 = \geq –5 $^{\circ}\text{C}$. In order to avoid strong selective pressure at an early backcross generation, we gave greater weight to the resistance traits than to the other characters. Hence the scores for the resistances were multiplied by a correction factor (cf) equal to 1.5/6, whereas the other traits were multiplied by a cf equal to 0.5/6. The EI was finally calculated following this formula: $\Sigma(\text{trait score} \times \text{cf}) \times 10$. The higher the index values, the more desirable were the hybrids. EI was calculated only for the hybrids for which all the evaluation data were available.

The various relevant correlations between ploidy level, fertility parameters, resistance traits and cmm-specific markers were evaluated by Spearman's coefficient of rank correlation.

Results

Mitotic analysis

The chromosome number of the PTH ranged from the tetraploid $2n=4x=48$ (two genotypes) to the hypertetraploid number $2n=4x+9=57$ (two genotypes) (Fig. 1, Table 2). Although 96% of hybrids had more than one extra chromosome, hybrids tended toward a low aneuploid level. Indeed, 71% had 48–53 chromosomes, whereas the others had 54, 55 and 57 chromosomes. The frequency distribution of PTH in ploidy classes was significantly different from that expected under random extra-chromosome distribution during meiosis of the pentaploid parent ($\chi^2=302.9$, $P<0.01$). Interestingly, the greatest deviation from the expected distribution occurred both at the tetraploid and pentaploid ends, where there was an excess of hybrids with 48, 50, 51, 53 chromosomes and a lack of hybrids with 55, 56, 58, 59 chromosomes, respectively. The expected mean, assuming a random transmission of extra chromosomes from the pentaploid MCPH 1, is $2n=54$. The observed mean of 52.4 represents a 27% loss of the extra genome in the hybrids analysed.

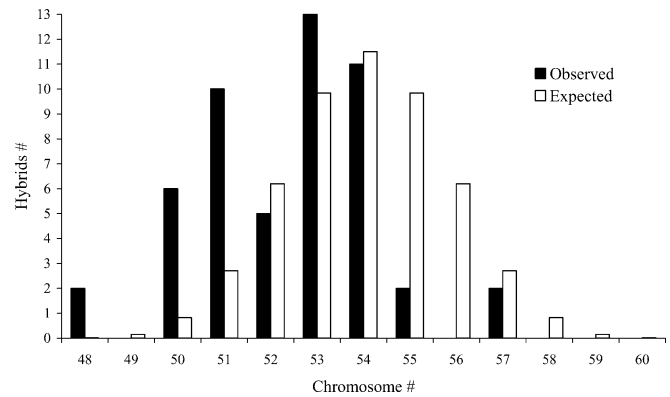


Fig. 1 Observed versus expected distribution of PTH hybrids in classes based on chromosome number

Fertility analysis

Data on our fertility analysis of the PTH are given in Table 1. In PTH \times tbr crosses, 80% of the hybrids analysed set fruits with seeds, with an average percentage of fruit set and number of seeds/fruit of 37% and 33%, respectively. Fruit set ranged from 0% (in nine cross combinations) to 100% (in four cross combinations). The number of seeds per fruit ranged from 8 to 90. Fruit set and seeds per fruit were significantly correlated ($r=0.36$, $P<0.05$). Differences between genotypes in terms of seeds per fruit were significant ($F=1.8$, $P<0.05$). On average, the germinability of seeds obtained from 23 PTH \times tbr crosses was 79%, with a range of 56% to 96%. For comparison, germinability of seeds from a tbr \times tbr cross was 100%. With respect to PTH male fertility, the average percentage of pollen stainability was 66%, with a range of 13% to 89%. The MCPH 1 parent displayed a pollen stainability of 62% (data not shown). In the reciprocal crosses, 59% of the tbr \times PTH combinations set fruits with seeds. On average, percentage of fruit set and the number of seeds/fruit were 21% and 39%, respectively. Fruit set ranged from 0 (in 17 cross combinations) to 100% (in one cross), whereas the number of seeds per fruit varied from 0 (in two crosses) to 121. Differences between genotypes in terms of number of seeds per fruit were highly significant ($F=12.7$, $P<0.01$). On average, the germinability of seeds from 16 tbr \times PTH cross combinations was 94% and ranged from 84% to 100%. The percentage of stainable pollen was significantly correlated with the number of seeds per fruit ($r=0.47$, $P<0.05$) but not with fruit set ($r=0.16$). A high significant correlation between fruit set and number of seeds per fruit was also found ($r=0.79$, $P<0.01$). Considering only crosses for which reciprocals were available, more PTH were fertile as female parents than as male parents (33 vs. 26), and on average, fruit set was higher in the PTH \times tbr crosses (36% vs. 23%). In contrast, the average number of seeds per fruit and seed germinability was higher in tbr \times PTH crosses. Differences between cross directions were not statistically significant for all of the parameters considered but it was for seed germinability ($F=24.2$, $P<0.01$). For both cross directions, chromosome number was not significantly correlated with

pollen stainability, fruit set, seeds per fruit and seed germinability (Table 3).

Tuber soft rot resistance and specific gravity of tubers

In the resistance test, significant differences between the 48 genotypes were found. The diameter of the rotted area ranged from 2.5 mm to 12.2 mm (not shown). Out of 48 hybrids tested, 14 were classified as resistant, 13 as intermediate and 21 as susceptible based on the scale adopted. The *tbr* control *Désirée* confirmed its susceptibility, with a diameter of decay of 10.6 mm. Table 2 gives detailed results for the hybrids and controls that were also analysed for their resistance to low temperatures. The parental genotypes MCPH 1 and *Blondy* had an intermediate resistance, with a diameter of decay of 4.6 mm and 4.9 mm, respectively, whereas the *tbr* control *Spunta* was susceptible. A random sample of hybrids (26) was evaluated again, and hybrids ranked similarly in the two tests ($r=0.56$, $P<0.01$). No significant correlation was found between hybrid chromosome number and resistance to Ecc ($r=0.02$; Table 3).

As for tuber specific gravity, a large variability was found for this trait, with values ranging from 1.044 (D 10) to 1.098 (D 2). Of the PTH hybrids analysed, 31% showed a specific gravity of tuber higher than 1.082, which is the minimum value requested for processing. The correlation between chromosome number and specific gravity was not significant ($r=0.04$), neither was the correlation between resistance to Ecc and specific gravity ($r=0.04$; Table 3).

Freezing tolerance

On average, the killing temperature of PTH hybrids under non-acclimated conditions was -2.8°C and ranged from -3.60 (D 16 and D 61) to -2.04°C (D 63) (Table 2). In general, the killing temperature of PTH under non-acclimated conditions did not differ from that of the standard varieties, and only two hybrids (D 16 and D 61) were close to MCPH 1 in their tolerance degree. Only six hybrids behaved better than *Spunta*. On average, AFT was -4.2°C and ranged from -6.05°C (D 23) to -2.63°C (D 7). The most interesting hybrids were D 23, D 54, D 46 and D 21 with an AFT of -6.05°C , -5.27°C , -5.11°C and -5.07°C , respectively. D 54 and D 23 also showed the highest cold acclimation capacity (3.21°C and 3.02°C ,

respectively) and relative acclimation capacity (161% and 152% for D 54 and D 23, respectively). The d/a value in non-acclimated PTH was 0.42 (Table 4), suggesting that the susceptible *tbr* phenotype was dominant for this trait. In contrast, killing temperatures of cold-acclimated PTH were distributed between the parental values and were often significantly higher than *tbr*, which is not able to cold-acclimate. The d/a value of -0.79 calculated for PTH indicated a high degree of dominance for cold acclimation ability. No significant correlation was found between hybrid chromosome number and freezing tolerance response ($r=0.024$ and 0.279 in non-acclimated and acclimated conditions, respectively; Table 3).

AFLP analysis

When all of the fragments scored for all of the primer combinations are used, the percentages of cmm-specific AFLPs ranged from 59% (D 58) to 91% (D 1), with an average of 75%. Figure 2 reports the recovering of *tbr* genome as a percentage of cmm-specific markers lost during backcrossing, starting from 100% present in F_1 hybrid B10 until the BC_2 PTH progeny. Following this criterion, we calculated that most of the hybrids recovered between 15% and 30% of the *tbr* genome.

The EI ranged from 18.3 (D 7, D 44 and D 58) to 35 (D56), with an average of 25.7. In Fig. 3, the 26 hybrids analysed are distributed according to their cmm-specific AFLP percentage and their EI value. Four hybrids (D 20, D 23, D 55 and D 56) had a higher EI and a lower cmm-specific AFLP percentage than the average for both characters.

Discussion

The hybrids that we have analysed here represent a skewed population in terms of chromosome number with a trend towards a low aneuploid level. $5x-4x$ progenies from other cross combinations also showed a similar trend (Carputo et al. 2003) with progeny means for chromosome number ranging from 50 ± 0.5 to 54 ± 0.7 . Our results represent indirect evidence of a non-random transmission of chromosomes during macrosperogenesis of the pentaploid parent MCPH 1. Vorsa (1988) suggested that the lack of fit between observed versus expected distribution can be attributed to the elimination of lagging chromo-

Table 1 Descriptive statistics for fertility parameters of 46 PTH hybrids used in crosses with *tbr* (SE standard error)

	Hybrids used ^a									
	♀ parent					♂ parent				
	Mean	SE	Minimum	Maximum	Median	Mean	SE	Minimum	Maximum	Median
Pollen stainability (%)	–	–	–	–	–	66	2	13	89	69
Fruit set (%)	37	5	0	100	33	21	4	0	100	14
Seeds/fruit (no.)	33	3	8	90	28	39	7	0	121	27
Germinability (%)	79	2	56	96	82	94	1	84	100	95

^a80% and 59% of the hybrids were fertile as female and male parents, respectively

Table 2 Chromosome number, killing temperature under non-acclimated (NAC) and acclimated (AC) conditions, cold acclimation capacity (ΔT), relative acclimation capacity and resistance to tuber soft rot of 26 PTH hybrids, their parents and *Spunta*

PTH hybrid	Chromosome no.	LT ₅₀ ^a (°C)		ΔT ^b (°C)	Relative acclimation capacity ^c (%)	Resistance to Ecc ^a [rotted area (mm)]
		NAC	AC			
D 1	53	-3.35c,d	-4.4d-f	1.01	51	4.7c,d
D 7	51	-2.54l,m	-2.63n	0.09	5	8.0a
D 10	51	-2.53l,m	-4.72b-e	2.19	110	4.8c,d
D 11	55	-2.30n,o	-4.21e-h	1.91	96	4.8c,d
D 13	51	-3.11e-g	-4.30d-f	1.19	60	4.7c-e
D 15	53	-2.99f-h	-4.09f-j	1.10	55	9.1a
D 16	54	-3.60a,b	-4.16e-l	0.56	28	3.4e-g
D 17	53	-2.58j-n	-3.12m	0.54	27	2.9f,g
D 18	53	-2.97f-h	-4.18f,g	1.21	61	5.2c
D 20	50	-2.68h-l	-4.14f-h	1.46	73	5.5c
D 21	51	-3.35c,d	-5.07b	1.72	86	3.0f,g
D 22	52	-3.25c-e	-4.91b-d	1.66	83	2.9f,g
D 23	50	-3.03f,g	-6.05b,c	3.02	152	2.5g
D 30	55	-2.20k-o	-3.33k-m	1.13	57	2.7g
D 37	51	-2.80g-l	-4.61b-f	1.81	91	7.9a
D 38	52	-2.31o	-3.82g-k	1.51	76	3.1f,g
D 44	48	-2.91g-l	-3.71g-l	0.8	40	6.6b
D 46	52	-3.08e-g	-5.11b	2.03	102	3.2f,g
D 51	53	-2.08g-j	-3.67i-k	1.59	80	3.8d-g
D 53	51	-2.09g,h	-3.93g-j	1.84	92	3.2f,g
D 54	-	-2.06i-n	-5.27b	3.21	161	2.7g
D 55	53	-3.01f-h	-4.13e-l	1.12	56	3.4e-g
D 56	-	-3.41e-g	-4.90b,c	1.76	88	4.2c-f
D 58	54	-2.85g-k	-3.97f-k	1.12	56	8.2a
D 61	53	-3.59a,b	-3.68h-l	0.09	5	5.4c
D 63	53	-2.04l-o	-3.09l-n	1.05	53	3.4e-g
cmm	24	-6.28a	-8.27a	1.99	100	- ^d
UP88-P5	24	-3.38b-d	-4.48c-f	1.1	55	-
MCPH 1	60	-3.68b	-4.30f	0.62	31	4.6c-e
<i>Blondy</i>	48	-2.42k-o	-3.31j-n	0.89	45	4.9c,d

^aMeans followed by same letter are not statistically different at $P < 0.05$

^b $\Delta T = AC$ killing temperature - NAC killing temperature

^cCalculated using the following formula $[(\Delta T \text{ hybrids}) / (\Delta T \text{ cmm})] \times 100$

^dCmm and UPP88-P5 were resistant and intermediate resistant, respectively, in previously screenings appropriated for mini-tubers

Table 3 Rank correlation coefficients between traits evaluated in PTH hybrids (NS not significant)

	Pollen stainability	Fruit set		Seeds/fruit		Germinability		Tuber specific gravity	Resistance to Ecc	Freezing tolerance ^a		cmm- specific AFLPs (%)
		♀	♂	♀	♂	♀	♂			NAFT	AFT	
Chromosome no.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pollen stainability		NS	NS	NS	0.47*	NS	NS	NS	NS	NS	NS	NS
Fruit set												
♀			NS	0.36*	NS	NS	NS	NS	NS	-0.71**	-0.70**	NS
♂				NS	0.79**	NS	0.67**	NS	NS	NS	NS	NS
Seeds/fruit												
♀					NS	NS	NS	NS	NS	NS	NS	NS
♂						NS	NS	NS	NS	NS	NS	NS
Germinability												
♀							NS	NS	NS	NS	NS	NS
♂								-0.78**	NS	NS	NS	NS
Tuber specific gravity									NS	NS	NS	NS
Resistance to Ecc										NS	NS	NS
NAFT											0.43*	NS
AFT												NS

* $P \leq 0.1$, ** $P \leq 0.05$

^aNAFT, non-acclimated freezing tolerance; AFT, acclimated freezing tolerance

Table 4 Degree of dominance for progeny means of NAFTA and AFT in each generation of the breeding scheme performed. Origin and pedigree of plant material are reported in Carputo et al. (2003)

Temperature (°C)				d/a^a
Resistant parent	Susceptible parent	Mid-parent value	Progeny	
Non-acclimated freezing tolerance (NAFT)				
cmm (-6.28)	UP88-P5 (-3.38)	-4.83	B10 (-4.10)	0.50
B10 (-4.10)	Wis 482 (-2.42)	-3.26	MCPH 1 (-3.68)	-0.50
MCPH 1 (-3.68)	Blondy (-2.42)	-3.05	PTH (-2.78)	0.42
Acclimated freezing tolerance (AFT)				
cmm (-8.27)	UP88-P5 (-4.48)	-6.40	B10 (-7.48)	-0.58
B10 (-7.48)	Wis 482 (-3.31)	-5.39	MCPH 1 (-4.30)	0.52
MCPH 1 (-4.30)	Blondy (-3.31)	-3.80	PTH (-4.19)	-0.79

$^a d/a = (\text{Progeny mean} - \text{mid-parent value}) / (\text{mid-parent value} - \text{low-parent value})$

somes or to selective mechanisms against aneuploidy at the gametophyte and/or post-zygotic stages. In any event, from the breeding standpoint, the recovery of genotypes with 48 chromosomes should be given attention as it suggests that selection against aneuploidy will skew the subsequent backcross generation further toward the tetraploid level. Tolerance to aneuploidy was also highly evident in the phenotype of the PTH hybrids, which did not show the morphological anomalies and decreasing vigour common to aneuploids of other species.

One of the main bottlenecks of aneuploid bridge generations is their becoming genetic “dead ends”. However, most of our hybrids were fertile in crosses with tbr both as the female and male parents, thereby allowing the production of offspring with two different cytoplasm types, depending on the direction of crosses. None of the correlations between aneuploidy and fertility parameters were significant, suggesting that the presence of extra chromosomes did not affect fertility. Khush (1973) suggested a buffering effect of polyploidy on aneuploidy, given that polyploids carry many duplications in their genomes and so further addition of single chromosomes may not alter their gene balance significantly. The reciprocal effect was not significant for fruit set percentage and number of seeds per fruit, indicating that macro- and microgametophytes have a similar tolerance to chromosome imbalance.

Interestingly, a number of hybrids displayed inheritance of valuable traits from cmm. Of great interest is the resistance of some PTH to Ecc. Due to the lack of effective chemical treatment, the constitution of Ecc-resistant varieties has become a major goal in potato breeding (Zimnoch-Guzowska and Lojkowska 1993). Aneuploidy did not influence resistance, and no significant correlation was found between chromosome number and soft rot resistance, indicating that resistance gene(s) may now reside in the potato genome. McGrath et al. (2002) reported on the introgression of soft rot resistance gene(s) from a *S. brevidens* (+) *S. tuberosum* somatic hybrid into the cultivated genome in early backcross generations. Screening for soft rot resistance of four BC generations, these authors found that the resistance level did not differ significantly between BC₂ populations and subsequent generations. Since no further segregation for Ecc resistance seemed to occur beyond the BC₂, introgression was taken to be stable in this generation.

With respect to freezing tolerance, we identified a number of hybrids able to acclimate down to around -5°C. Some of these exhibited an acclimation capacity higher than 2°C, which is the hardiness level of some pure hardy species (Chen et al. 1999). Cold acclimation ability is one of the major goals for potato breeders in the Mediterranean area, where early potato is a high-profit crop. In this region potato is planted from the late fall onwards, and frost events are generally “announced” by cool days which

Fig. 2 Estimated genome composition of 26 PTH hybrids. Estimation was based on the percentage of cmm-specific AFLPs still present after two backcross generations. The loss of cmm-specific AFLPs was viewed as a complementary gain of tbr genome. The chromosome number of each hybrid is reported at the top of each bar

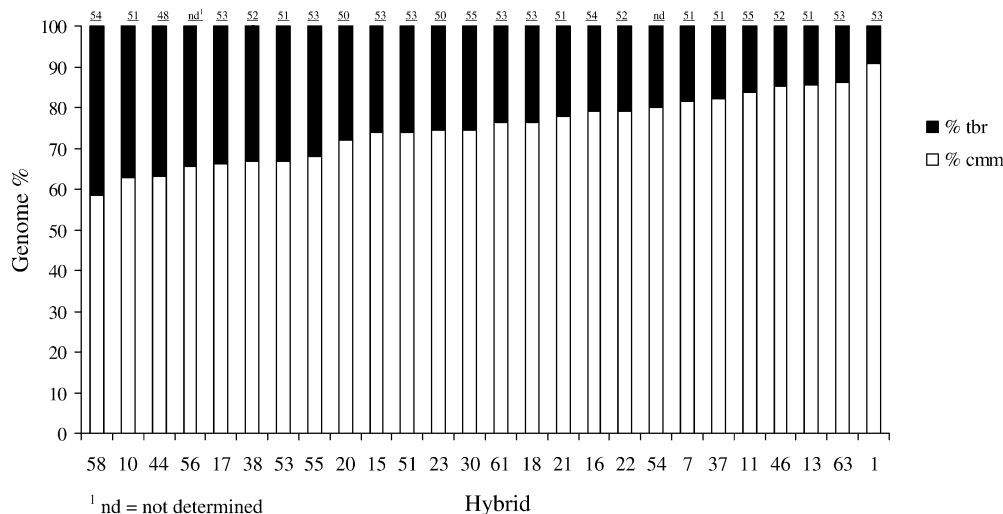
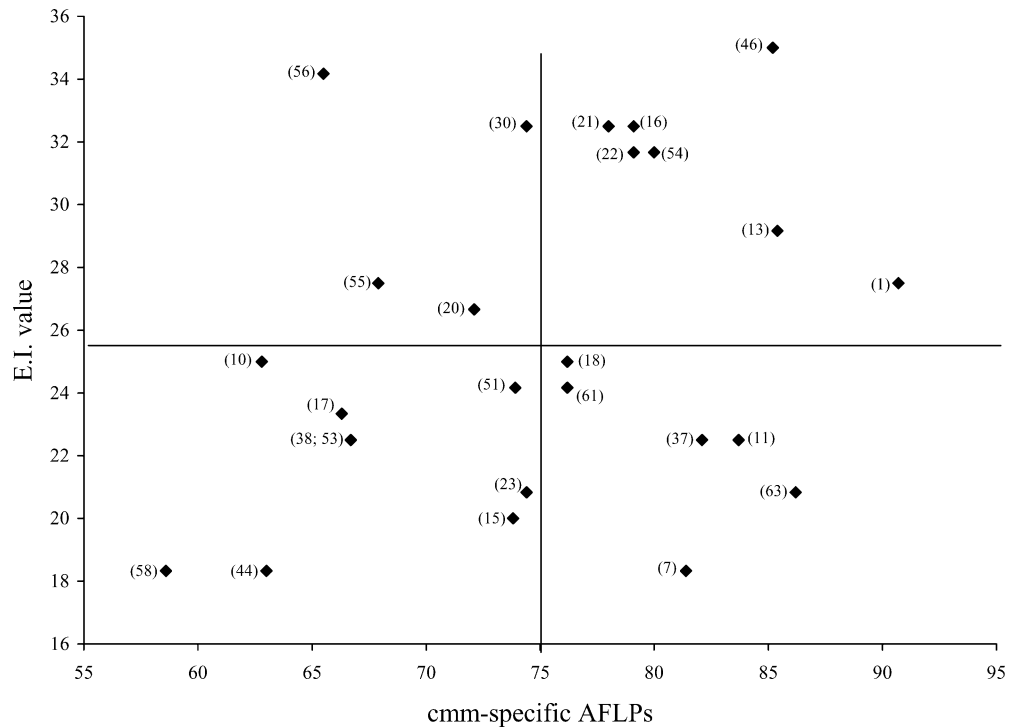


Fig. 3 Scatter diagram of 26 PTH hybrids according to their percentage of cmm-specific AFLPs and E.I. Hybrid identity is reported in *parenthesis*



allow the plants to cold acclimate. Screening for NAFTA and AFT confirmed that, in potato, AFT is easier to introgress than NAFTA (Stone et al. 1993). In fact, on the basis of the *d/a* value calculated, the cold-acclimating capacity derived from the wild cmm seemed to be dominant over the lack of ability of the cultivated *tbr* to acclimate to cold. In contrast, the degree of dominance for freezing tolerance per se indicated a recessive inheritance pattern for this trait. Our results confirm that chromosome number did not affect freezing tolerance, whereas interesting correlations were found between resistance to low temperature and some fertility parameters, suggesting the possible presence of linkage or pleiotropic effects. However, a larger sample size and specific genetic analysis are required to better study the relationship between genes involved in freezing tolerance and fertility.

Breeding schemes involving wild species are known to be complicated by the transmission of unwanted traits from the wild donor into the cultivated gene pool. For instance, cmm may transmit high glycoalkaloid content, long stolons and a lack of adaptation to long days. Therefore, time-consuming backcrossing, evaluation and selection are required to restore the improved cultivated phenotypes. Phenotypic selection may be hampered by the fact that the targeted exotic genes are usually transferred together with flanking segments containing many additional and often unwanted genes from the wild donor parent (Gepts 2002). This linkage drag is one of the main factors that has limited the use of wild *Solanum* species to date (Pavek and Corsini 2001). Molecular markers represent a powerful aid in backcross breeding, especially when markers linked to the target gene(s) are available. Alternatively, genetic markers may speed up the restoration of the cultivated phenotype by selecting for genotypes

with a minimum linkage drag near introgressed regions and/or against markers from the wild parent outside the region carrying the target gene(s). In this study, cmm-specific markers were used to monitor the degree of wild genome still present in the hybrids. Thus, selection was carried out against the wild genome rather than for target gene(s). In particular, selection was performed according to a two-stage scheme based on conventional phenotypic selection followed by estimation of wild genome content so as to identify hybrids combining noteworthy traits with a low wild genome content. AFLPs were chosen because they allowed the identification of a large number of cmm-specific markers in each BC generation (Barone et al. 2001). Molecular analysis provided evidence of a still high wild genome content in the hybrids that was not comparable with that predicted for a conventional BC₂ generation. In addition, it allowed the identification of some hybrids with a higher evaluation index and a lower wild genome content than the average for both characters. To speed up breeding efforts, these hybrids are being used for further evaluations. The selection strategy used here seems to be encouraging because some of the 5x × 4x hybrids, already used as parental lines in backcrosses, gave progenies with good agronomic and quality traits and a much lower wild genome content (Carputo et al. 2002).

We believe that three main factors emerge from this research. First, aneuploidy does not influence either fertility or noteworthy agronomic traits, thereby refuting the commonly field opinion that imbalanced chromosome numbers may create dead-ends. Second, hybrids combining useful traits and a low degree of wild genome have been identified. Lastly, assisted selection can be effective even when performed against the wild genome content.

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