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Resistance to Frost and Tuber Soft Rot in Near-Pentaploid *Solanum tuberosum*–*S. commersonii* Hybrids

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The objectives of the present study were to evaluate the tolerance to low temperatures and tuber soft rot in sexual near-pentaploid hybrids between incongruent 2x (1EBN) *Solanum commersonii* (CMM) and 4x (4EBN) *S. tuberosum* (TBR). For freezing resistance, killing temperatures both under non-acclimated and under acclimated conditions were determined using the ion leakage procedure. Values for the hybrids were distributed between the wild and cultivated parental values. Some hybrids displayed an acclimation capacity close to 2.5°C, typical of hardy species. Artificial inoculation of tubers with *Pectobacterium carotovorum* ssp. *carotovorum* (formerly *Erwinia carotovora* ssp. *carotovora*) provided evidence of variability in disease response. Highly resistant hybrids were identified. After conventional phenotypic selection, wild genome content was estimated based on the presence of CMM-specific AFLP fragments. Seven primer combinations were used (*Eco*-AGG/*Mse*-CAA; *Eco*-ACC/*Mse*-CAT; *Eco*-ACT/*Mse*-CAC; *Eco*-ACT/*Mse*-CAG; *Eco*-ACT/*Mse*-CAA; *Eco*-ACT/*Mse*-CAT; *Eco*-AGG/*Mse*-CAG). The percentages of CMM-specific AFLPs ranged from 4.3% to 56.7%, with an average value of 28.1%. AFLP analysis was employed for the selection of the hybrids to be used for further breeding objectives.

Key Words: *Pectobacterium carotovorum*, endosperm balance number (EBN), assisted selection, resistance to low temperatures, germplasm introgression, molecular markers.

Introduction

The wild relatives of the tetraploid (2n = 4x = 48) cultivated potato *Solanum tuberosum* (TBR) provide a rich, unique and diverse source of genetic variation for potato breeding (Bradshaw *et al*. 2006). Genes for resistance to environmental stresses, and tuber characteristics such as high solid content, good chipping quality and skin color can be easily found in these species. Ploidy in wild *Solanum* species ranges from diploidy (2n = 2x = 24) to hexaploidy (2n = 6x = 72). Since most of these species are diploid, they can be easily crossed with haploids (2n = 2x = 24) derived from TBR varieties (Carputo and Barone 2005). Hybrids developed display a 50% wild genome, and can be used in sexual polyploidization crossing schemes to restore the chromosome number of cultivated potato, and gradually reduce the wild genome content. This breeding approach has the advantage of offering disomic rather than tetrasomic inheritance patterns, and requires a smaller population size compared to conventional breeding at the tetraploid level (Peloquin *et al.* 1999). However, post-zygotic barriers sometimes hamper the sexual exploitation of noteworthy diploid species such as *S. bulbocastanum, S. brachistotrichum, S. cardiophyllum* and *S. commersonii* (CMM). These barriers lead to endosperm degeneration in the hybrid seed. Johnston *et al*. (1980) advanced the endosperm balance number (EBN) hypothesis to explain the failure of interspecific crosses in *Solanum*. They postulated that each species has a specific EBN value (ranging from 1 to 4), and that a 2 : 1 maternal to paternal EBN ratio in the hybrid endosperm is a necessary condition for normal development. Since several sexually isolated diploid species have an EBN of 1 (Johnston and Hanneman 1982), crosses with TBR haploids (2EBN) and 2x (2EBN) species cannot be easily made due to EBN imbalance.

Among the 1EBN wild species, CMM displays several resistance and quality traits (Hanneman and Bamberg 1986), including resistance to frost and capacity for cold acclimation after exposure to low temperatures (Palta and Simon 1993). Both traits are very important, since temperatures below 0°C are often a major factor for the decrease of tuber yield, and since cultivated potato is frost-sensitive and lacks the capacity for cold acclimation. Stone *et al*. (1993) reported that frost resistance and acclimation capacity are independent traits with partial recessive inheritance. They also provided evidence that relatively few genes are involved in

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their genetic control. Noteworthy is the resistance of CMM to tuber soft rot caused by *Pectobacterium carotovorum* ssp. *carotovorum* (formerly *Erwinia carotovora* ssp. *carotovora*). This bacterium may hamper potato cultivation under several environmental conditions, causing losses in the field and during tuber storage. The control of soft rot is difficult due to the wide range of hosts and of temperatures at which *Pectobacterium* spp. can grow and because tubers may be latently infected by bacteria located in the vascular system or in suberized lenticels. Unfortunately, the frequency and degree of resistance are very low in cultivated potato, and there is no effective method of chemical control (Zimnoch-Guzowska *et al*. 2006).

To overcome the sexual isolation of CMM, Carputo *et al*. (1997) developed a breeding scheme based on the production of triploid and pentaploid bridge crosses. The strategy was based on doubling the chromosome number of CMM, and on the production of 3x(2EBN) bridges derived from 4x × 2x crosses between 4x(2EBN) CMM and 2x(2EBN) genotypes. Since the triploids produced 2n eggs, they could be used in 3x × 4x crosses with TBR to obtain (near)pentaploid bridges displaying a high level of aneuploidy (Carputo 2003). The unique ploidy of the genotypes produced made this material suitable for broadening our understanding of the cytological, genetic and reproductive behavior of genotypes with odd ploidies. This seems very interesting in the potato where a ploidy series (including triploids and pentaploids) naturally occurs and, especially at the pentaploid level, only scant information is available. The objective of the present study was to characterize (near)pentaploid bridges derived from 3x × 4x crosses for resistance to low temperatures and tuber soft rot in CMM, in order to provide evidence that they could be used in conventional breeding. To assist selection, AFLP analysis was carried out to indirectly estimate the wild genome content of each hybrid.

Materials and Methods

Plant material included 31 (near)pentaploid bridges generated from 3x × 4x crosses, as reported by Carputo *et al*. (1997). Their somatic chromosome number ranged from hypo-pentaploid 2n = 5x – 6 = 54, to hyper-pentaploid 2n = 5x + 7 = 67, with the euploid pentaploid 2n = 5x = 60 class being predominant (Carputo 2003).

Clonally propagated hybrids were evaluated for freezing tolerance before and after cold acclimation through the electrolyte leakage procedure described by Carputo *et al*. (2003). Plants from the CMM and TBR parents (PI243503 and cv. Blondy, respectively) were also included in the test. In brief, four clonally propagated plants from each genotype were cultured in a growth chamber under cool white fluorescent lamps (350–400 $\mu mol\ m^{-2}\ s^{-1}$) at 18–20°C (dark/light) for the non-acclimation studies. For the cold acclimation studies, two plants per genotype were transferred to a cold room (4–2°C, light/dark) at 100 $\mu mol\ m^{-2}\ s^{-1}$ for two weeks. Mature expanded leaves were put in culture tubes and submerged in a glycol bath at 0°C. Three leaves (replications) per genotype were used in each temperature treatment. Temperature was lowered by 0.5°C every 30 minutes. The control treatment consisted of three replicating per genotype kept on ice at 0°C. After 30 minutes at the desired freezing temperature, the tubes were placed on ice to thaw overnight. The freezing tolerance (non-acclimated, NAFT; acclimated, ACFT) for each genotype was determined by calculating the temperature at 50% of freezing injury (LT_{50}), according to the logistic model described by Janácek and Prásil (1991). Statistical differences between the LT_{50s} of the genotypes tested were calculated using the program LT_{50} version 2.1 (Janácek and Prásil 1991). Mean separation was performed using the Duncan's Multiple Range Test. The difference between ACFT and NAFT was defined as acclimation capacity.

Genotypes that produced a suitable amount of healthy tubers were screened for the resistance to tuber soft rot caused by *Pectobacterium carotovorum* ssp. *carotovorum* (PCC). Ten tubers from 21 (near) pentaploids and their TBR parent Blondy were used for this evaluation. Strain 009 of PCC was provided by the International Potato Center, Lima, Peru. Tubers were inoculated according to the procedure described by Iovene *et al*. (2004). Three to five holes (2 cm deep) were made in each tuber using a sterilized drill. One hole was inoculated with distilled water as a control, the others with 20 µl of bacterial suspension (10^7 cfu/ml). Individual holes were considered as replications for this study. After 72 hours of incubation at 24°C in a dew chamber, the diameter of the rotted area was measured. Following the scale reported by Iovene *et al*. (2004), genotypes with an average diameter of rotted area smaller than 4 mm were classified into resistant, those with a decay diameter between 4 and 6 mm into intermediate, and those with a diameter of rotted area larger than 6 mm into susceptible. Data for the decay rotten diameter in the inoculated holes were used for analysis of variance. Means separation was performed using the Duncan's Multiple Range Test.

A resistance index (RI) was calculated for each genotype by assigning to each resistance trait an arbitrary scale (Iovene *et al*. 2004): Resistance to PCC, 1 = diameter of rotted area ≥8; 2 = diameter of rotted area: 6–8 mm; 3 = diameter of rotted area: 4–6 mm; 4 = diameter of rotted area ≤4 mm. Non-acclimated freezing tolerance (NAFT), 1 = ≤TBR (°C); 2 = TBR (°C)/–3°C; 3 = –3.1°C/–4°C; 4 = ≥–4.1°C. Acclimated freezing tolerance (ACFT), 1 = ≤TBR (°C); 2 = TBR (°C)/–4°C; 3 = –4.1°C/–5°C; 4 = ≥–5.1°C. In this scale, the score 1≤TBR indicates a value numerically (and not statistically) less than that of TBR. The RI value of each genotype was finally calculated as Σ (trait score). The higher the RI value, the more desirable were the hybrids. RI was calculated only for the hybrids for which all the evaluation data were available.

AFLP analysis was carried out according to the method described by Vos *et al.* (1995) and using the commercially available AFLP kit and protocol (Gibco-BRL AFLP analysis

System I, Life Technologies, Gaithersburg, MD), in which *Eco*RI and *Mse*I are the restriction enzymes. Out of 31 hybrids, 2 were not included in the analysis because they did not produce clear and scorable profiles with the primer combinations used. Plants from the CMM and TBR parents (PI243503 and cv. Blondy, respectively) were also included. For selective amplification, seven combinations of the primers previously selected (Barone *et al*. 2001) were used (*Eco*-AGG/*Mse*-CAA; *Eco*-ACC/*Mse*-CAT; *Eco*-ACT/*Mse*-CAC; *Eco*-ACT/*Mse*-CAG; *Eco*-ACT/*Mse*-CAA; *Eco*-ACT/*Mse*-CAT; *Eco*-AGG/*Mse*-CAG). A fluorometric method was applied for detecting AFLP fragments with a higher resolution than that of conventional radio-detection techniques (Schwarz *et al*. 2000). *Eco*RI primers were labelled with 6-carbozy-fluorescein (6-FAM), while the *Mse*I primers were not labelled. AFLP fragments were electophoretically separated on 6% denaturing polyacrylamide gels and the fluorescence was detected with a Typhoon 9210 scanner (Amersham). AFLP images were then analyzed with ImageQuant TL software, v2003.02 (Amersham Biosciences 2002), and by visual inspection. For each genotype, polymorphic fragments were recorded as present (1) or absent (0). Ambiguous fragments were scored as missing (9). The percentage of TBR- and CMM-specific markers was calculated as follows:

% CMM/TBR-specific AFLPs = (no. of CMM/TBR-specific markers observed in each hybrid/total no. of CMM/TBR-specific markers analysed) × 100.

Results

Freezing tolerance of non-acclimated and acclimated genotypes is listed in Table 1. Significant differences among the genotypes were observed both in terms of NAFT ($F = 11.48$, $P < 0.01$) and ACFT ($F = 11.51$, $P < 0.01$). CMM showed high NAFT and ACFT values, which were significantly different from those of all the other genotypes (−6.28°C and −8.17°C, respectively). The near-pentaploid hybrids displayed killing temperatures distributed between the parental values, both with and without acclimation. On average, killing temperatures ranged from −3.30°C to −4.60°C under non-acclimated and acclimated conditions, respectively. The NAFT values of the tested hybrids ranged from −2.27°C (MCPH30) to −5.02°C (MCPH22), those of ACFT from −3.50°C (MCPH34) to −6.78°C (MCPH22). Cold acclimation treatment resulted in an increased freezing tolerance of 1.89°C in CMM, while in the TBR control, there was only a slight change in the freezing tolerance (0.91°C), within the range of the non-acclimating genotypes. As for the near-pentaploid genotypes, the average acclimation capacity was 1.3°C, with a range from 0.14 to 2.76. In most of the hybrids, the acclimation capacity was higher than that of the sensitive parent but lower than that of CMM. However, eleven genotypes (T1, MCPH1, MCPH5, MCPH6, MCPH13, MCPH17, MCPH21, MCPH22, MCPH28, MCPH30, MCPH33) showed an acclimation capacity as high as that of CMM. A significant positive correlation was found between NAFT and ACFT ($r_s = 0.38$, $P < 0.05$) (Fig. 1), whereas no significant correlation was found between the somatic chromosome number and NAFT ($r_s = -0.04$), ACFT ($r_s = -0.13$).

Results from the screening test for tuber soft rot resistance are presented in Table 1. Significant differences were observed among the genotypes tested ($F = 25.62$, $P < 0.01$). A wide range in susceptibility was displayed in the genotypes tested, with a diameter of rotted area ranging from 15.7 mm (MCPH33) to 3.2 mm (MCPH21). Out of 21 hybrids tested, 2 (MCPH21 and MCPH13) were classified into resistant, according to the scale adopted. As for the others, 7 were classified into intermediate (diameter of rotted area: 4–6 mm) and 12 into susceptible (diameter of rotted area larger than 6 mm). The somatic chromosome number of the near-pentaploid hybrids was positively correlated with the diameter of the rotted area ($r_s = 0.46$, $P < 0.05$). The relationships between the resistance to tuber soft rot, NAFT and ACFT are shown in Figure 1. No significant correlation was found between soft rot and either NAFT ($r_s = -0.01$) or ACFT ($r_s = -0.12$).

Considering the fragments scored for all the primer combinations used, the total number of CMM-specific markers was 164, and that of TBR-specific markers 94. Percentages of CMM-specific AFLPs ranged from 4.3% (MCPH 24) to 56.7% (MCPH 20), with an average value of 28.1% (data not shown). Figure 1 indicates the number of TBR and CMM-specific AFLP markers for each hybrid. Values of CMM-specific markers ranged from 7 (MCPH 24) to 86 (MCPH 13), those of TBR-specific markers from 6 (MCPH 24) to 63 (MCPH 35). Great similarity (~0.86) was found among MCPH15, MCPH18 and MCPH24, which showed a low content of CMM and TBR bands. In contrast, MCPH20, MCPH 21 and MCPH 23 showed the lowest similarity index (~0.53) (data not shown). No AFLP marker associated with the resistance traits evaluated in this study was detected. The RI ranged from 6 (MCPH3 and MCPH25) to 11 (MCPH2 and MCPH21), with an average value of 8.1. Figure 2 shows the scatter diagram of 20 near-pentaploid hybrids based on RI values and the number of CMM-specific AFLPs. Genotypes with the highest RI values (11, MCPH21 and MCPH2; 10, MCPH16) exhibited 62, 48 and 31 CMM-specific AFLPs, respectively.

Discussion

Although odd-ploidy genotypes are often referred to as genetic “dead ends”, previous results demonstrated that several near-pentaploid bridges used in the present study were fertile in crosses with TBR (Carputo 2003). In addition, in spite of being aneuploid, the plants did not generally show phenotypic aberrations or vigor reduction common to aneuploids of other species and resembled *S. tuberosum* in growth habit (Caruso 2005). Thus, if such plants were to display useful traits from CMM, they could be normally used in

Table 1. Chromosome number, killing temperature in non acclimated (NAFT) and acclimated (ACFT) conditions, cold acclimation capacity (ΔT), and resistance to tuber soft rot of 31 near-pentaploid hybrids between *S. commersonii* (CMM, PI 243503) and *S. tuberosum* (TBR, cv. Blondy).

Near-pentaploid hybrid	Chromosome no.	LT50[a] (°C)		ΔT[b] (°C)	Resistance to PCC[a] (diameter rotted area, mm)
		NAFT	ACFT		
T1	60	–2.95 m–q	–4.93 e–i	1.98	14.1 a
P5	60	–3.78 d	–4.28 m–q	0.50	7.5 c
1	62	–2.32 tu	–4.60 n–r	2.28	na
2	na[c]	–4.36 c	–6.02 b–d	1.66	4.7 d–f
3	60	–3.56 d–g	–4.02 o–s	0.46	11.5 b
5	60	–4.45 c	–6.75 bc	2.30	na
6	67	–2.88 n–r	–5.17 e–h	2.29	8.1 c
7	67	–3.26 f–m	–4.48 i–o	1.22	6.8 c–e
11	58	–3.08 h–o	–4.74 g–m	1.66	5.3 d–f
13	57	–2.39 s–u	–4.81 f–l	2.42	3.3 f
14	63	–2.43 r–u	–3.99 o–t	1.56	4.7 ef
15	58	–3.36 f–l	–3.98 p–t	0.62	5.3 d–f
16	58	–3.76 de	–5.09 e–h	1.33	4.3 ef
17	58	–2.78 o–r	–5.41 c–g	2.63	na
18	na	–4.21 c	–4.35 i–p	0.14	6.9 cd
19	64	–3.02 l–p	–4.23 n–r	1.21	6.1 ce
20	62	–3.42 e–h	–4.39 i–p	0.97	5.9 ce
21	60	–3.37 e–l	–5.34 d–g	1.97	3.2 f
22	58	–5.02 b	–6.78 b	2.76	11.2 b
23	58	–3.63 d–f	–4.71 g–n	1.08	na
24	60	–3.14 g–n	–4.05 o–s	0.91	na
25	64	–3.08 h–p	–3.69 r–u	0.61	14.1 a
27	62	–2.88 n–q	–3.62 tu	0.74	na
28	64	–3.58 d–f	–5.72 c–f	2.14	na
29	na	–2.66 q–t	–3.82 r–u	1.16	7.5 c
30	60	–2.27 u	–4.49 h–o	2.22	na
31	64	–3.60 d–f	–3.91 p–u	0.31	5.9 c–e
32	64	–3.13 g–o	–3.55 u	0.42	7.6 c
33	66	–3.36 f–l	–5.38 d–g	2.02	15.7 a
34	54	–2.77 or	–3.50 u	0.73	na
35	na	–2.66 p–t	–3.72 v	1.06	na
CMM	24	–6.28 a	–8.17 a	1.89	–[d]
TBR	48	–2.42 r–u	–3.33 u–v	0.91	8.2 c

[a] means followed by same letter are not statistically different at $P < 0.05$.

[b] ΔT = ACFT killing temperature – NAFT killing temperature.

[c] na, not available.

[d] CMM was classified resistant following screening tests on mini-tubers.

breeding programs. The resistance traits that we evaluated in the present study are major objectives for potato breeding. We identified a number of hybrids with freezing resistance and capacity for acclimation down to around −7°C. Some of them exhibited an acclimation capacity at above 2°C, that corresponds to the hardiness level of some pure hardy species (Chen *et al*. 1999). In our experiments, freezing resistance and acclimation capacity were positively correlated, and both traits were found in the same clones (e.g. MCPH5 and MCPH22). Our results provided evidence that the chromosome number did not affect either the freezing tolerance or acclimation capacity, suggesting the absence of gene dosage effects. It is possible that the extra-chromosomes present in our near-pentaploids did not carry the loci involved in the genetic control of these traits. In contrast, previous results from the screening of triploid, pentaploid and tetraploid CMM-TBR hybrids suggested the existence of a genome dosage effect, by which a larger number of genomes from CMM increased the resistances (Carputo *et al*. 2003).

Among the useful traits exhibited by CMM, the resistance to PCC is of primary importance. Indeed, up to now, attempts at chemical control of potato bacterial diseases had been unsuccessful (Zimnoch-Guzowska *et al*. 2006). Therefore, the development of PCC-resistant varieties has become a major objective in potato breeding. Although the resistance to PCC is polygenic (Zimnoch-Guzowska *et al*. 2000), we were able to identify hybrids with a high to moderate resistance. The somatic chromosome number influenced the disease response, as indicated by a significant positive correlation coefficient between the chromosome number and the

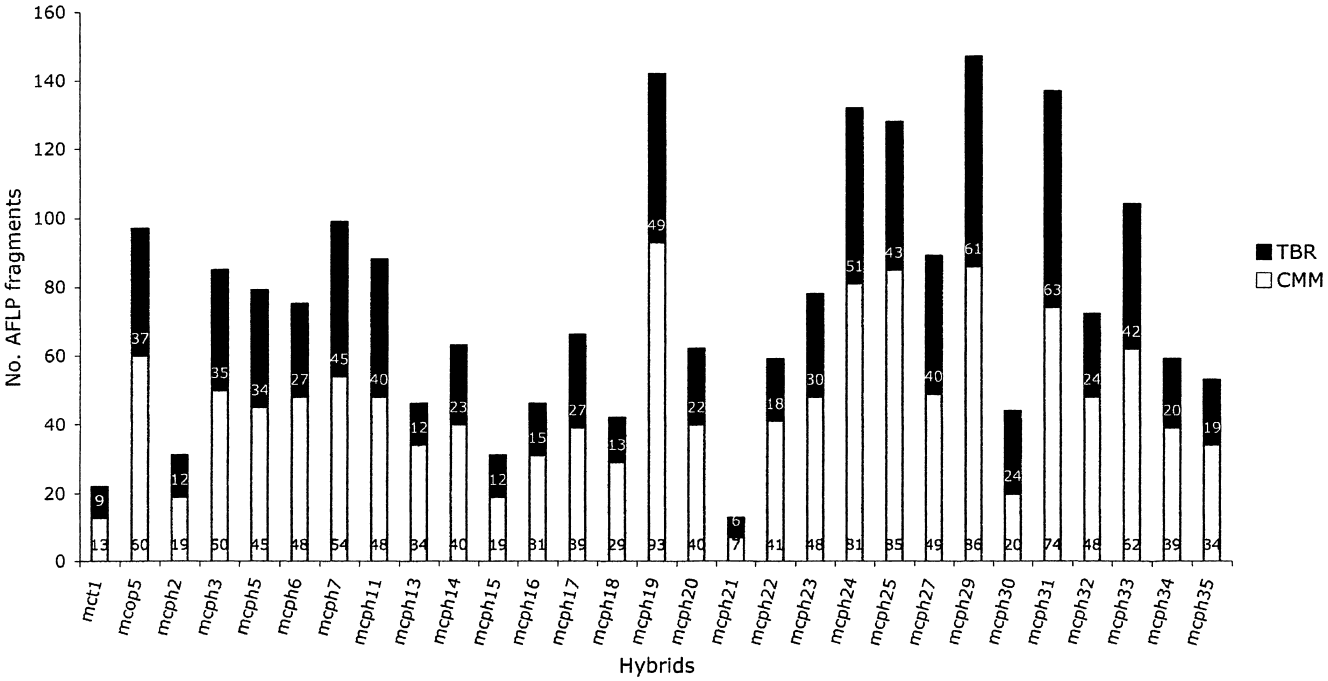

Fig. 1. Number of *S. tuberosum* (TBR) and *S. commersonii* (CMM)-specific AFLP fragments found in near-pentaploid CMM-TBR hybrids. Seven primer combinations were used (for details see Materials and Methods).

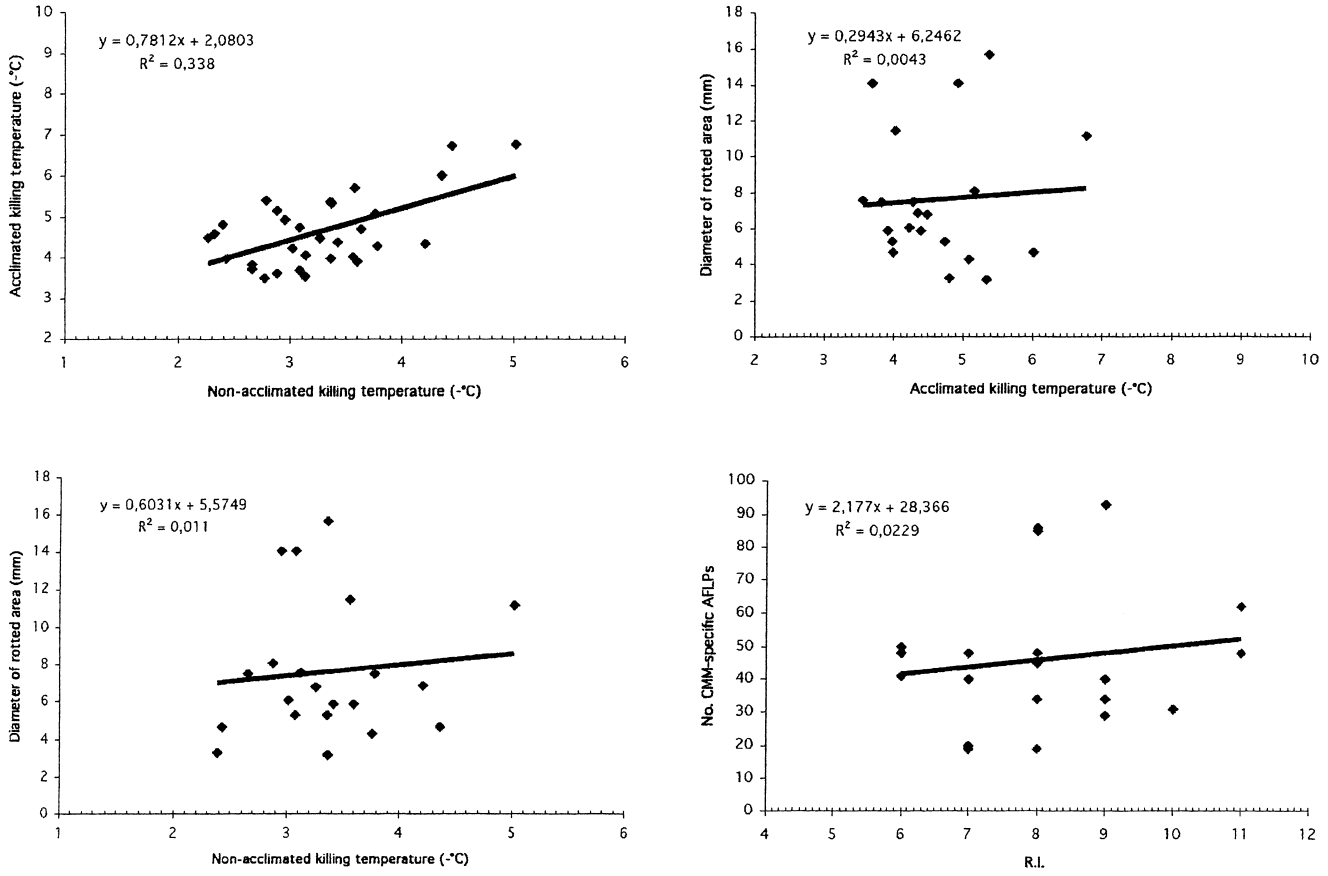

Fig. 2. Relationship between resistance traits estimated in near-pentaploid hybrids. Traits considered are as follows: killing temperature under acclimated and non-acclimated conditions and diameter of rotted area after inoculation of *Pectobacterium carotovorum* ssp. *carotovorum*. The relationship between the number of *S. commersonii*-specific AFLPs and resistance index (RI) is also shown.

diameter of the rotted area. It may be suggested that due to a non-random transmission of chromosomes of the 3x parent generating our near-pentaploids, extra-chromosomes were mainly of the TBR-type and that the presence of susceptible alleles at higher dosage influenced the response to the artificial inoculation with PCC. It can also be postulated that, in hybrids with a higher chromosome imbalance, competition among the different regulatory elements of parental chromosomes may reduce the expression of genes from the resistant parent. We were able to identify a hybrid material combining noteworthy values for both resistances. In MCPH13 and MCPH21, for example, a diameter of rotted area < 4 mm was associated with ACFT values of −4.81°C and −5.34°C, respectively. This is very important considering that these traits are not associated. Therefore, if backcrosses were made with selection for acclimation capacity, resistance to tuber soft rot could be easily deselected.

Interspecific hybridization is often complicated by the transmission of chromosome segments linked to useful genes, but that harbor unwanted genes from the wild parent (Gepts 2002). Pavek and Corsini (2001) reported that linkage drag is probably the main factor that has limited the use of wild *Solanum* species so far. Therefore, time-consuming breeding efforts are required to restore the improved cultivated phenotypes. Molecular markers are very useful for breeders, especially when markers linked to target gene(s) are available. Mullins *et al*. (2006) have recently pointed out that few instances of molecular marker-assisted selection have been reported in potato. They suggested that this was largely due to the tetraploid level of the plant and to the polygenic control of many traits of interest. The identification of DNA traits associated with specific genes based on the use of markers randomly distributed in the genome appears to be very promising. We have attempted to use unmapped AFLPs to speed up selection. No association was detected between CMM-specific AFLPs and either resistance to tuber soft rot or to low temperatures. A more detailed genetic study should be carried out for the identification of loci exerting major effects on the aforementioned resistances. Chagué *et al.* (1997), for instance, demonstrated the feasibility of "Bulk Segregant Analysis" for quantitative inherited traits by selecting individuals with extremely contrasting phenotypes to form the two pools used in the analysis. Molecular markers can also be used to indirectly estimate the degree of wild genome still present in the hybrids (Barone 2004). Therefore, the RI of each hybrid was plotted against the number of CMM-specific AFLPs. Hybrids with the highest value of CMM-specific AFLPs did not show the highest RIs, suggesting a lack of correlation between analyzed traits and AFLPs, and the possibility to select against high wild genome content without deselecting for resistances. As for the hybrids with a low CMM-specific AFLP content, it should be pointed out that the low value may indicate that their AFLP markers did not represent the 12 chromosomes comprising the basic chromosome set of CMM. Therefore the results obtained in the latter hybrids reflect possible bias in AFLP derivation from a few CMM chromosomes rather than from all 12.

In conclusion, manipulations of ploidy and EBN resulted in a new genetic material potentially valuable for potato breeding. We were able to use resistance genes from sexually isolated CMM in near-pentaploid hybrids. In addition, even in a small sample size, we could identify genotypes combining a capacity for cold acclimation and resistance to *Pectobacterium* spp. (e.g. MCPH13 and MCPH21). Preliminary studies also indicated that these hybrids display a high yield potential under long-day conditions (Caruso 2005). They are fertile, and have already been used in crosses with *S. tuberosum*. Rapid progress in obtaining a larger number of 48-chromosome genotypes is expected after further backcrosses.

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Literature cited

Barone, A. (2004) Molecular marker assisted selection for potato breeding. Am. J. Potato Res. 81: 111–117.

Barone, A., A. Sebastiano, D. Carputo, F. della Rocca and L. Frusciante (2001) Molecular marker-assisted introgression of the wild *Solanum commersonii* genome into the cultivated *S. tuberosum* gene pool. Theor. Appl. Genet. 102: 900–907.

Bradshaw, J.E., G.J. Bryan and G. Ramsay (2006) Genetic resources (including wild and cultivated *Solanum* species) and progress in their utilisation in potato breeding. Potato Res. 49: 49–65.

Carputo, D. (2003) Cytological and breeding behavior of pentaploids derived from 3x × 4x crosses in potato. Theor. Appl. Genet. 106: 883–888.

Carputo, D., A. Barone, T. Cardi, A. Sebastiano, L. Frusciante and S.J. Peloquin (1997) Endosperm Balance Number manipulation for direct *in vivo* germplasm introgression to potato from a sexually isolated relative (*Solanum commersonii* Dun.). Proc. Natl. Acad. Sci. USA 94: 12013–12017.

Carputo, D., A. Terra, A. Barone, F. Esposito, V. Fogliano, L. Monti and L. Frusciante (2003) Glycoalkaloids and acclimation capacity of hybrids between *Solanum tuberosum* and the incongruent hardy species *S. commersonii*. Theor. Appl. Genet. 107: 1187–1194.

Carputo, D. and A. Barone (2005) Ploidy manipulation in potato through sexual hybridization. Ann. Appl. Biol. 146: 71–79.

Caruso, I. (2005) Caratterizzazione e selezione assistita di ibridi pentaploidi e aneuploidi-pentaploidi tra *Solanum tuberosum* (2n = 4x = 48, 4EBN) e *S. commersonii* (2n = 2x = 24, 1EBN). M.S. Thesis, University of Naples "Federico II".

Chagué, V., J.C. Mercier, M. Guénard, A. de Courcel and F. Vedel (1997) Identification of RAPD markers linked to a locus involved in quantitative resistance to TYLCV in tomato by

bulked segregant analysis. Theor. Appl. Genet. 95: 671–677.

Chen, Y.K.H., J.P. Palta and J.B. Bamberg (1999) Freezing tolerance and tuber production in selfed and backcross progenies derived from somatic hybrids between *Solanum tuberosum* L. and *S. commersonii* Dun. Theor. Appl. Genet. 99: 100–107.

Gepts, P. (2002) A comparison between crop domestication, classical plant breeding, and genetic engineering. Crop Sci. 42: 1780–1790.

Hanneman, R.E. Jr. and J.B. Bamberg (1986) Inventory of tuber-bearing *Solanum* species. Research Bullettin 533, University of Wisconsin, Madison, Wisconsin, 216.

Iovene, M., A. Barone, L. Frusciante, L. Monti and D. Carputo (2004) Selection for aneuploid *Solanum commersonii-S. tuberosum* hybrids combining low wild genome content and resistance traits. Theor. Appl. Genet. 109: 1139–1146.

Janácek, J. and I. Prásil (1991) Quantification of plant frost injury by nonlinear fitting of an S-shaped function. Cryo-Lett. 12: 47–52.

Johnston, S.A., T.M. den Nijs, S.J. Peloquin and R.E. Jr. Hanneman (1980) The significance of genic balance to endosperm development in interspecific crosses. Theor. Appl. Genet. 57: 5–9.

Johnston, S.A. and R.E. Hanneman, Jr. (1982) Manipulations of Endosperm Balance Number overcome crossing barriers between diploid *Solanum* species. Science 217: 446–448.

Mullins, E., D. Milbourne, C. Petti, B.M. Doyle-Prestwich and C. Meade (2006) Potato in the age of biotechnology. Trends Plant Sci. 11: 254–260.

Palta, J.P. and G. Simon (1993) Breeding potential for improvement of freezing stress resistance: genetic separation of freezing tolerance, freezing avoidance, and capacity to cold acclimate. *In* "Advances in plant cold hardiness" Li, P.H. and L. Christersson (eds.), CRC Press, Boca Raton, Florida. p. 299–310.

Pavek, J.J. and D.L. Corsini (2001) Utilization of potato genetic resources in variety development. Amer. J. Potato Res. 78: 433–441.

Peloquin, S.J., L. Boiteaux and D. Carputo (1999) Meiotic mutants in the potato—valuable variants. Genetics 153: 1493–1499.

Schwarz, G., M. Herz, X.Q. Huang, W. Michalek, A. Jahoor, G. Wenzel and V. Mohler (2000) Application of fluorescence based semi-automated AFLP analysis in barley and wheat. Theor. Appl. Genet. 100: 545–551.

Stone, J.M., J.P. Palta, J.B. Bamberg, L.S. Weiss and J.F. Harbage (1993) Inheritance of freezing resistance in tuber-bearing *Solanum* species: Evidence for independent genetic control of non-acclimated freezing tolerance and cold acclimation capacity. Proc. Natl. Acad. Sci. USA 90: 7869–7873.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acid Res. 23: 4407–4414.

Zimnoch-Guzowska, E., W. Marczewski, R. Lebecka, B. Flis, R. Schafer-Pregl, F. Salamini and C. Gebhardt (2000) QTL analysis of new sources of resistance to *Erwinia carotovora* ssp. *atroseptica* in potato done by AFLP, RFLP and resistance-gene-like markers. Crop Sci. 40: 1156–1167.

Zimnoch-Guzowska, E., E. Lojkowska and M. Pérombelon (2006) Resistance to bacterial pathogens. *In* "Genetic improvement of Solanaceous crops, Vol. 1: Potato" Razdan, M.K. and A.K. Matto (eds), Science Publishers Inc., Enfield, USA. p. 339–395.