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ORIGINAL PAPER

Nuclear and cytoplasmic genome components of *Solanum tuberosum* + *S. chacoense* somatic hybrids and three SSR alleles related to bacterial wilt resistance

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Abstract The somatic hybrids were derived previously from protoplast fusion between *Solanum tuberosum* and *S. chacoense* to gain the bacterial wilt resistance from the wild species. The genome components analysis in the present research was to clarify the nuclear and cytoplasmic composition of the hybrids, to explore the molecular markers associated with the resistance, and provide information for better use of these hybrids in potato breeding. One hundred and eight nuclear SSR markers and five cytoplasmic specific primers polymorphic between the fusion parents were used to detect the genome components of 44 somatic hybrids. The bacterial wilt resistance was assessed thrice by inoculating the in vitro plants with a bacterial suspension of race 1. The disease index, relative disease index, and resistance level were assigned to each hybrid, which were further analyzed in relation to the molecular markers for elucidating the potential genetic base of the resistance. All of the 317 parental unique nuclear SSR alleles appeared in the somatic hybrids with some variations in the number of bands detected. Nearly 80 % of the hybrids randomly showed the chloroplast pattern of one parent, and most of the hybrids exhibited a fused mitochondrial DNA pattern. One hundred and nine specific SSR alleles of *S. chacoense* were analyzed for their relationship with the disease index of the hybrids, and three alleles were identified to be significantly associated with the resistance. Selection for the resistant SSR alleles of *S. chacoense* may increase the possibility of producing resistant pedigrees.

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Introduction

Wild *Solanum* species have many useful and desirable traits lacking in cultivated potato *Solanum tuberosum* ($2n = 4x = 48$). However, incompatibility of interspecific sexual hybridization may hamper their utilization in potato breeding (Johnston et al. 1980). Plant somatic hybridization can transfer large amount of genomic DNA or organelle DNA between the sexually incompatible species, which has been considered a potential avenue for using wild germplasms in modern potato breeding. Some intraspecific and interspecific somatic hybrids have been obtained in *Solanum* for transferring of useful genes, creating the new nuclear-cytoplasmic germplasms, and inducing variability in plastidial and mitochondrial DNA (Lovene et al. 2007; Greplová et al. 2008; Tiwari et al. 2010; Patel et al. 2011; Sarkar et al. 2011).

Bacterial wilt caused by *Ralstonia solanacearum* is a severe and devastating disease in most tropical, subtropical, and warm temperate areas and even in some cool temperate regions (Hayward et al. 1998). It affects more than 200 plant species distributed in more than 50 families, particularly in the potato and tomato (Hayward 1994). The most effective method to better control potato bacterial wilt

is to generate resistant varieties, but lack of resistance in spp. *tuberosum* has bogged down the potato breeding progress in almost last half century (Hermsen 1994). It has been documented that some wild or related cultivated species are resistant or highly tolerant to bacterial wilt, such as *Solanum chacoense* and *S. sparsipilum* (Hawkes 1994). To introgress valuable genes into the *S. tuberosum* gene pool more efficiently, protoplast fusion has been adopted by others (Laferriere et al. 1999; Fock et al. 2001; Kim-Lee et al. 2005), and somatic hybrids of *S. tuberosum* + *S. chacoense* possibly resistant to bacterial wilt have been obtained in our laboratory (Cai et al. 2004).

Somatic hybridization can generate extensive nuclear and cytoplasmic fusion in the hybrids with potential incorporation of chloroplast and mitochondria from both fusion parents (Davey et al. 2005). For efficient use of somatic hybrids, characterization of the genetic composition of hybrids is an important prerequisite (Lovene et al. 2007). However, little information is available about the genomic components of the somatic hybrids in potato and how the genomic combinations relate to the performance of the hybrids and their implementation in a breeding program. Especially for bacterial wilt resistance introduced from a wild species without fully understand of the inheritance, the genome component analysis attaches more importance to elucidate the genetic base of the resistance. Modern molecular tools now make it more feasible than ever before with conventional genetics, to develop specific parental genomic markers and/or probes and to link them with desirable traits.

For the nuclear components analysis, molecular technologies and the genomic in situ hybridization (GISH) were used to study the genomic dosage of the parental genomes in the hybrids (Lovene et al. 2007; Thieme et al. 2010). It is also critical to look into recombinations of mitochondria (mt) and chloroplast (cp) DNA, since protoplast fusion produces variations not only at the nuclear level but also in cytoplasmic DNA (Lovene et al. 2007). The most common method used for cytoplasmic composition analysis is Southern analysis with specific mt and cp probes (Lössl et al. 1999; Bastia et al. 2000; Trabelsi et al. 2005). In past decade, several "universal primers" homologous to conserved sequences of mtDNA and cpDNA and amplifying highly variable regions have been developed (Scotti et al. 2003, 2007). Compared with Southern blot, this approach is cheaper and less time-consuming.

In this paper, we report the genomic components of somatic hybrids between *S. tuberosum* and *S. chacoense* identified by specific nuclear and cytoplasmic DNA markers. The potential nuclear SSR alleles associated with bacterial wilt resistance of the wild parent were identified.

Materials and methods

Somatic hybrids

Forty-four somatic hybrids were produced from the protoplast fusion between *S. tuberosum* and *S. chacoense*. The procedure for obtaining the somatic hybrids was described by Cai et al. (2004). The *S. tuberosum* fusion parent $3^{\#}$ ($2n = 4x = 48$) is susceptible to bacterial wilt and is a somatically-doubled of a dihaploid of cultivar Zhongshu 2, which is susceptible to bacterial wilt; while the wild species fusion parent C9701 is a clone of *S. chacoense* ($2n = 2x = 24$) selected for resistance to bacterial wilt. The hybrids were numbered as 3 C*i*-*j* (*i* represents the *i*th callus, *j* represents the *j*th plant regenerated from callus *i*). For example, 3C1-1 is the first plant of the first callus from $3^{\#}$ + C9701. The ploidy of the hybrids and the fusion parents were measured by Guo et al. (2010). The somatic hybrids were cultured in vitro on MS medium (Murashige and Skoog 1962) supplemented with 4 % sucrose and 0.7 % agar at 20 ± 1 °C with a 16 h light/8 h dark cycle.

Molecular analysis

Total DNA was extracted from leaves of in vitro plants of the hybrids using the CTAB method as described by Dellaporta et al. (1983). The polymerase chain reaction (PCR) was performed in a 20-μL reaction mixture containing 30 ng template DNA, 1.5 mM $MgCl_2$, 1 × PCR buffer (50 mM KCl, 10 mM Tris–HCl, pH 8.3), 0.2 mM dNTP mix, 0.12 μM of the forward and reverse primers, and 0.5 U of Taq polymerase. Reactions were performed in 96-well plates in a Bio-RAD C1000^{TM} thermal cycler.

One hundred and seventy-three simple sequence repeat (SSR) primer pairs, obtained from CIP database (http://research.cip.cgiar.org/IPD/SSR-primer), Feingold et al. (2005) and Ghislain et al. (2009), were tested for the nuclear polymorphism between the fusion parents, and 108 primer pairs (Supplementary Table S1) were selected because they could amplify the parental unique bands clearly and repeatedly. The PCR procedure was: 3 min initial denaturation at 95 °C, 35 cycles of amplification for 30 s at 95 °C, 30 s at annealing temperature (see Supplementary Table S1 for specific temperature for each pair of primers) and 1 min at 72 °C, and extension for 5 min at 72 °C. Finally, the products were kept at 10 °C until use. The indication of a range in the annealing temperature column of Table S1 denotes the use of a touchdown profile (Don et al. 1991). In this case, the annealing temperature used in the first cycle was the higher temperature of the range, with a 0.5 °C decrease per cycle until the lower temperature was reached; additional cycles at the lower

annealing temperature were added to complete 35 cycles in total. The nuclear SSR PCR products were run on 9 % polyacrylamide gels and silver-stained. Band size on a fingerprinting panel was determined by comparison with a 72-bp DNA ladder. The amplified fragments were named as "primer name + band size".

12 SSR primers and 1 universal primer of chloroplast DNA (cpDNA) and 17 specific primers of mitochondrial DNA (mtDNA) (Supplementary Table S2) were used in this study to detect the cytoplasmic components of the somatic hybrids and their stability during long-term culture in vitro. The cpDNA detection was carried out in 2003, 2005, and 2010, and the mtDNA detection was carried out in 2009 and 2011. Success of amplification was checked under UV light after electrophoresis in 1 % agarose gels run in 1× TAE and stained with ethidium bromide (EB).

Assessment of resistance to bacterial wilt

The strain of *Ralstonia Solanacearum* (race 1, biovar 3), isolated from infected potato plants in southwest Hubei of China, was used to inoculate the fusion parents and the somatic hybrids. Tests to evaluate the resistance level to bacterial wilt were performed using in vitro plants. These were cultured at 20 ± 1 °C with 16 h/day light of 60 μmol m^{-2} s^{-1} and about 60 % relative humidity for 3 weeks before inoculation by pouring a bacterial solution (1×10^7 colony forming units per mL onto the plant's hurt roots injured by a sterile knife). Control plants were inoculated with sterile water. The tests for bacterial resistance were carried out on three occasions using 27 plants per test distributed between three replications. Each replication of nine plants was placed in a culture chamber. Two weeks after inoculation, the plants were assessed for disease symptoms using a disease score (DS) ranging from 0 to 4 (0 no wilted leaves; 1 up to 25 % wilted; 2 26–50 % wilted; 3 51–75 % wilted; 4 more than 75 % leaves wilted) (Kim-Lee et al. 2005). A disease index (DI) was calculated for each plant material tested following the method of Winstead and Kelman (1952) as DI = Σ (number of the plants with a specific DS × DS)/(total number of inoculated plants × 4). For comparison between the tests, a relative disease index (RDI) was calculated as RDI = (DI of susceptible control − DI of the tested plant material)/(DI of susceptible control) × 100 %. Based on the RDI value, the resistance was classed into four levels: resistant (R, RDI > 70 %), medium resistant (MR, RDI = 40–70 %), medium susceptible (MS, RDI = 20–39 %), and susceptible (S, RDI < 20 %) (Liang and He 1999).

Statistical analysis

The SSR alleles data were scored as "1" (band present) or "0" (band absent). The significance of the differences among the means of disease index was analyzed by Duncan's test using the SAS8.1 program. The Bivariate Correlate Analysis in the SPSS18.0 program was used to evaluate the relationship between molecular makers and bacterial wilt resistance of the somatic hybrids, and the Spearman correlation coefficient was used to estimate the significance of the correlation.

Results

Nuclear genome components

The nuclear genome components of the somatic hybrids were clarified with selected SSR markers. In this study, 108 SSRs yielded clear and repeatable polymorphic bands between the two fusion parents. Except for STG0017, the other 107 SSRs had chromosome location information which distributed from chromosome 1 to 12 and averaged 8.9 SSRs on each chromosome (Supplementary Table S1). In total, these polymorphic SSRs detected 317 alleles in the fusion parents. Of them, 159 alleles were unique in the cultivated parent $3^{\#}$, and 109 alleles were unique in the wild parent C9701; while the other 49 alleles were common bands to both parents (Table 1). The average number of diverse alleles detected by the SSRs was 2.9 and ranged from 1 to 7 among individuals. These results indicate that the selected SSRs can detect a relatively wide genetic diversity in the fusion parents and are suitable for the analysis of the nuclear genome components of their derived somatic hybrids.

All of the 44 hybrids contained alleles from both parents but with different genome dosages. A total of 12,576 bands were amplified in the somatic hybrids with an average of 285.8 in each hybrid (Table 1). All of the 268 unique alleles detected in the fusion parents appeared in the somatic hybrids with some variations among the hybrids, suggesting the protoplast fusion could allow sufficient nuclear genome combination with diverse individual cases. On average, 143.1 polymorphic alleles from the cultivated parent $3^{\#}$ and 91.1 polymorphic alleles from the wild parent C9701 were present in the hybrids accounting for 90.02 and 83.59 %, respectively, in terms of the proportion of number of the parental bands detected in the hybrids. Although the coefficient of variation for each parent's polymorphic alleles appeared in the somatic hybrids was <10 %, some big differences were observed in individual hybrids, for example, two somatic hybrids (3C35-3 and 3C39-1) contained 158 of the 159 unique alleles of the cultivated parent $3^{\#}$, but only 120 and 123 alleles could be detected in 3C8-3 and 3C15-1, respectively. Similarly, the hybrid 3C36-1 had 102 of the 109 alleles of the wild parent C9701, whereas only 79 alleles could be found in 3C7-1

Table 1 Nuclear components of the somatic hybrids analyzed by SSR markers

Genotypes	Ploidy level[a]	No. of Unique alleles of 3# (%[b])	No. of Unique alleles of C9701 (%[b])	No. of common bands of 3# and C9701	No. of novel bands
3#	4x	159	0	49	0
C9701	2x	0	109	49	0
3C1-1	6x	138 (86.79)	85 (77.98)	48	2
3C1-2	6x	143 (89.94)	89 (81.65)	48	2
3C1-3	6x	141 (88.68)	87 (79.82)	48	2
3C1-4	5x, 6x	149 (93.71)	85 (77.98)	48	2
3C1-5	6x	150 (94.33)	92 (84.40)	48	2
3C2-1	6x	148 (93.08)	93 (85.32)	48	2
3C2-3	6x	149 (93.71)	91 (83.49)	48	2
3C3-1	NT	150 (94.34)	91 (83.49)	48	2
3C3-3	6x	138 (86.79)	96 (88.07)	46	2
3C3-4	6x	145 (91.19)	82 (75.23)	48	2
3C3-5	4x, 6x	155 (97.48)	93 (85.32)	47	2
3C3-6	6x	157 (98.74)	90 (82.56)	48	2
3C3-7	6x	150 (94.34)	93 (85.32)	49	2
3C3-8	5x, 6x	152 (95.60)	88 (80.73)	48	2
3C6-2	5x	131 (82.39)	84 (77.06)	48	2
3C7-1	6x, 8x	135 (84.91)	79 (72.48)	47	2
3C8-1	5x	134 (84.28)	93 (85.32)	48	2
3C8-2	6x	138 (86.79)	90 (82.57)	47	2
3C8-3	6x	120 (75.47)	91 (83.49)	46	2
3C8-4	5x	132 (83.01)	94 (86.24)	46	2
3C10-1	5x, 6x	136 (85.53)	89 (81.65)	48	2
3C10-2	7x	132 (83.02)	93 (85.32)	46	2
3C12-1	6x	134 (84.28)	96 (88.07)	47	2
3C12-2	6x	146 (91.82)	92 (84.40)	47	2
3C14-1	6x	144 (90.57)	91 (83.49)	46	2
3C15-1	6x	123 (77.36)	95 (87.16)	45	2
3C18-1	6x	154 (96.86)	93 (85.32)	48	2
3C19-1	6x	147 (92.45)	83 (76.15)	47	2
3C21-1	5x, 7x	131 (82.39)	82 (75.23)	46	2
3C25-3	4x, 6x	125 (78.62)	97 (88.99)	48	2
3C27-1	6x	144 (90.57)	95 (87.16)	48	2
3C28-1	5x	142 (89.31)	87 (79.81)	48	2
3C29-2	6x	149 (93.71)	90 (82.57)	48	2
3C30-1	6x	151 (94.97)	92 (84.40)	48	2
3C30-2	6x	156 (98.11)	99 (90.83)	48	2
3C31-1	6x	154 (96.86)	94 (86.24)	48	2
3C33-1	4x, 6x	154 (96.86)	95 (87.16)	47	2
3C33-2	6x	150 (94.34)	94 (86.24)	48	2
3C34-1	4x, 6x	128 (80.50)	90 (82.57)	48	0
3C35-1	6x	139 (87.42)	91 (83.49)	49	2
3C35-2	5x, 6x	145 (91.19)	92 (84.40)	46	2
3C35-3	5x, 6x	158 (99.37)	97 (88.99)	47	2
3C36-1	5x, 6x	143 (89.94)	102 (93.58)	45	2
3C39-1	NT	158 (99.37)	94 (86.24)	47	2
Total		6,298	4,009	2,183	

Table 1 continued

Genotypes	Ploidy level[a]	No. of Unique alleles of 3# (%[b])	No. of Unique alleles of C9701 (%[b])	No. of common bands of 3# and C9701	No. of novel bands
Average of hybrids		143.1 (90.02)	91.1 (83.59)	47.4	1.95
CV (%)		6.8	5.1	2	15
Minimum		120 (75.54)	79 (72.48)	45	0
Maximum		158 (99.37)	102 (93.58)	49	2

[a] *NT* not tested

[b] The percent of polymorphic alleles of the parent in the hybrids

(Table 1). The genomic dosage of 3# and C9701 in the hybrids was further analyzed after arcsine transformation of the percent of polymorphic alleles of each fusion parent appeared in the hybrids by paired samples *t* test. A significant difference ($P = 0.048$) was detected between the parents, suggesting that the hybrids retained a higher proportion of nuclear DNA dosage from cultivated parent than wild parent.

The results further demonstrated that the genetic variations that occurred in the somatic hybrids may be mainly from different combination rates of the polymorphic alleles. For example, the cultivated parent's specific alleles TISP.264 and TISP.251 existed in 10 hybrids, whereas another 51 alleles were present in all of the 44 hybrids. Similarly, none of hybrids showed the specific alleles STM0001.126 and STI0025.114 from C9701, while all the hybrids gained 29 alleles. In contrast to polymorphic alleles, the hybrids possessed nearly all 49 common bands (average 47.7) which may not eventually reflect an occurrence of chromosome combination.

Appearance of new bands and loss of polymorphic bands were also observed in present research. When using the SSR marker STM2022, there were two novel bands, not showed in either of the fusion parents (Fig. 1), amplified in 43 of the somatic hybrids but not in 3C34-1. The average absent percentage of the 317 unique alleles was 12.59 % in the hybrids. However, the SSR alleles presented in Table 2 were those been absent in more than 50 % of the somatic hybrids, speculating that the absence of SSR alleles in somatic hybrids did not occur randomly, this genetic variation was prone to happen in some special locations.

Cytoplasmic genome components

To determine the cytoplasmic components of the *S. tuberosum* + *S. chacoense* somatic hybrids, the polymorphisms between the parental cpDNAs and mtDNAs were detected using a set of gene-specific primer pairs. Low levels of cytoplasmic DNA polymorphisms between the fusion parents were observed in this study. All of the 13 cpDNA primer pairs used could amplify clear bands, but only NTCP-9 generated fragment length polymorphism between the fusion parents; while the other 12 primers did not show any differences. The fragment amplified by NTCP-9 was 300 bp in cultivated parent 3# and 240 bp in wild parent C9701 (Fig. 2a). These two fragments were sequenced and compared with the sequences in NCBI, which showed that the DNA sequence of 3# had 96 % homology with the chloroplast DNA sequence of *S. lycopersicon* (GenBank accession number AM087200.3) and the DNA sequence of C9701 had 94 % homology with the chloroplast DNA sequence of *S. bulbocastanum* (GenBank accession number DQ347958). Although all the 17 mtDNA primer pairs could generate amplifications, only four primer pairs (Primer8, 10, 15, and 16) were polymorphic between 3# and C9701 (Fig. 2b). Primer8 and Primer10 were specific primers for C9701 amplifying fragments of about 1,800 and 1,600 bp, respectively. Primer15 and Primer16 were specific primers for 3# which yielded fragments of about 1,000 and 400 bp, respectively. These four DNA fragments showed more than 95 % similarity with the mitochondrial DNA sequences, the former two were homology with the mitochondrial DNA sequence of

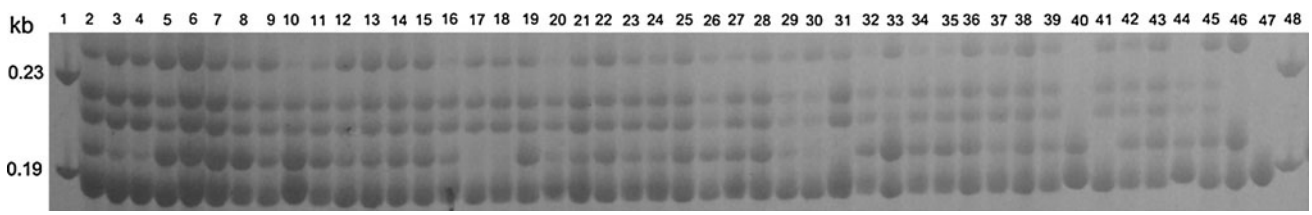

Fig. 1 SSR analysis of both fusion parents and their somatic hybrids amplified with the primer STM2022. *Lanes* 1 and 48 DNA size marker, 2–47 somatic hybrids, 46 cultivated parent 3#, 47 wild parent C9701

Table 2 The SSR alleles which were absent in more than 50 % of the somatic hybrids

SSR alleles	The absent numbers in the hybrids (%)	Chromosome location[a]
STM2020.173	26 (59.09)	1
STG0006.152	39 (88.63)	2
STG0006.138	38 (86.36)	2
STG0010.160	22 (50.00)	3
STI0026.213	27 (61.36)	4
STI0001.210	33 (75.00)	4
STI0049.159	31 (70.45)	5
STI0049.155	27 (61.36)	5
STI0049.138	43 (97.73)	5
Stac.229	24 (54.55)	5
STM0001.126	44 (100)	6
STM0001.97	43 (97.72)	6
STI0025.114	44 (100)	7
STM1105.108	33 (75.00)	8
TISP.264	34 (77.27)	10
TISP.251	34 (77.27)	10
STM0051.89	39 (88.64)	10
STM2012.241	39 (88.64)	10
TISP.274	36 (81.82)	10
TISP.244	35 (79.55)	10
STG0003.130	32 (72.73)	12

S. commersonii (GenBank accession number AJ582177.2) and the later two were homology with the mitochondrial DNA of *S. tuberosum* (GenBank accession number AF095277.l). These results demonstrated that the fragments amplified with the cpDNA primer NTCP-9, and the four mtDNA primers certainly were cytoplasmic DNA sequences and could be used to detect the cpDNA or mtDNA types of the somatic hybrids.

The results indicated that most of the somatic hybrids (33 out of 42 hybrids tested) showed the chloroplast pattern of one parent (Table 3; Fig. 2a). Among these, 17 showed the band specific to $3^{\#}$, and 16 detected the band from C9701. In SIX hybrids, bands from both parents were detected at least once in the three tests. But the results were variable either because of complex recombination of cpDNA from both parents or simple recombination of cpDNA from only one parent. The rest three were not constant by changing from one parent type to another.

In contrast to the cpDNA, 41 out of the 44 hybrids showed a fused mtDNA pattern (Fig. 2b) and at least one locus were detected by primers specific for each parent. Three hybrids (3C3-3, 3C8-2, and 3C34-1) had no mtDNA from the cultivated parent $3^{\#}$. In seven hybrids, the mtDNA pattern changed between the tests in 2009 and 2011 represented by missing of one locus except for 3C8-1, which showed the absence of Primer10 and addition of Primer15. However, these variations did not obviously affect the components of the mitochondrial genome in the somatic hybrids, since only one hybrid (3C8-4) changed from the wild parent type to the fused type as detected by Primer16 and one hybrid (3C10-2) changed from the fused type to the cultivated parent type and showed no band from Primer10. Further analysis demonstrated that the locus detected by Primer10 had the highest fusion rate in the somatic hybrids (95 %), followed by the loci detected by Primers 15 and 16 (89 % for each); while Primer8 had the lowest rate (55 %).

Evaluation of resistance to bacterial wilt

Resistance to bacterial wilt was tested thrice for each somatic hybrid and the fusion parents. The inoculation results tabulated in Table 4 show the mean disease index (DI) and relative disease index (RDI) and the resistance level assigned based on the RDI value. The wild parent C9701 appeared to be resistant or tolerant (Fig. 3a) to race 1 and had a low DI value (0.286). In contrast, the cultivated parent $3^{\#}$ was susceptible (Fig. 3h) to race 1 of bacterial wilt. It wilted rapidly and easily and had a DI of 0.986. The DI values of the 44 somatic hybrids ranged from 0.301 to 1.0 with RDI varying between 0 and 0.695. The DIs of seven somatic hybrids did not differ significantly from the resistant wild species C9701 (e.g. Fig. 3b–d) and were assessed as medium resistant (MR) according to their RDIs of 69.5–43.5 %. Six hybrids were considered medium susceptible (MS) with DIs between 0.667 and 0.787 and

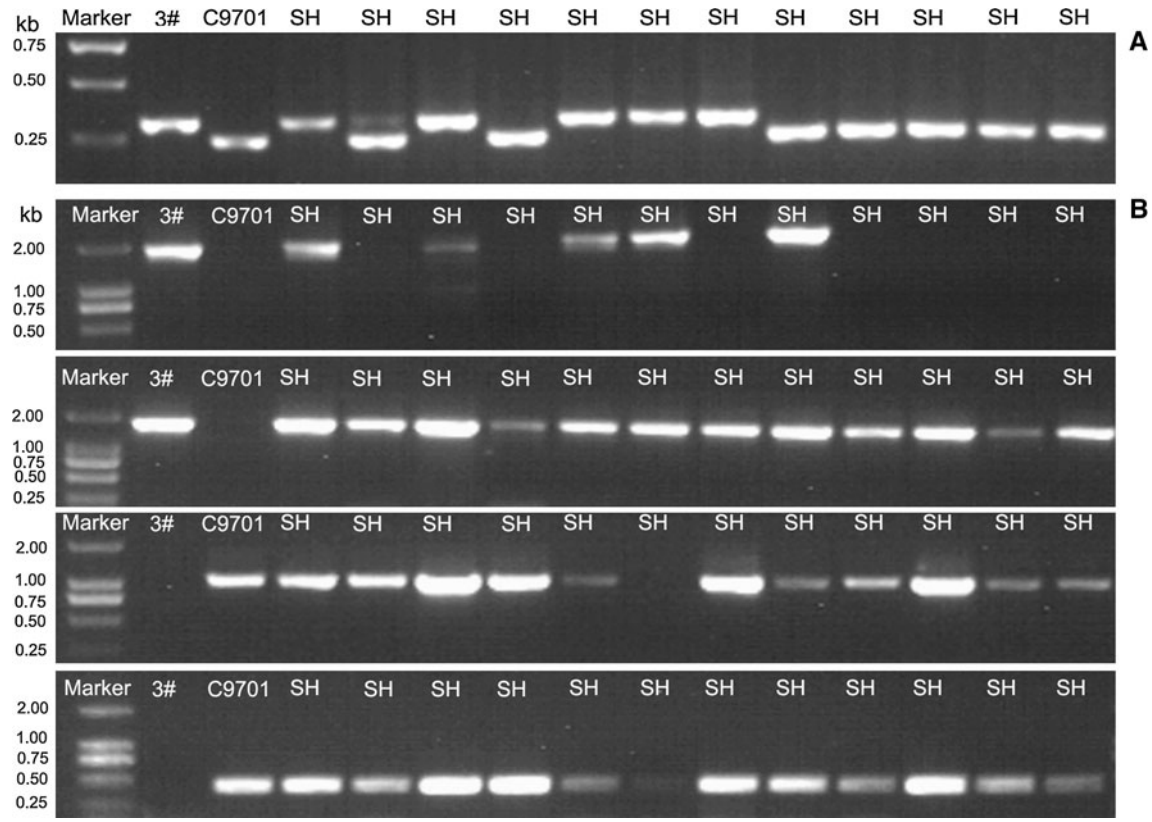

Fig. 2 PCR analysis of both fusion parents and their somatic hybrids using the cpDNA and mtDNA primer pairs. *SH* somatic hybrids, **a** cpDNA banding pattern amplified using the primer pair NTCP-9, **b** mtDNA banding pattern amplified with the primer pairs Primer8, Primer10, Primer15 and Primer16 from the *top* to the *bottom* orderly

RDIs between 32.4 and 20.2 %. The other 31 somatic hybrids were susceptible (e.g., Fig. 3e–g) with higher DI values and RDIs of <20 %. Further analysis showed variation in resistance among the hybrids formed from the same callus. For example, 3C3-4 was medium resistant to bacterial wilt with DI of 0.301; whereas 3C3-8, regenerated from the same callus (callus 3) was susceptible with a DI value up to 0.991. Polymorphisms in nuclear and cytoplasmic DNAs in the hybrids formed from the same callus imply that callus 3 is a chimera formed not from a single fused cell. The results suggest that different genome components affect the degree of resistance to bacterial wilt transmitted from the wild species in somatic hybrids.

Correlation analysis of molecular markers and resistance to bacterial wilt in potato somatic hybrids

To identify the SSR alleles associated with bacterial wilt resistance in potato, all of the 109 unique SSR alleles from C9701 were analyzed in relation to the resistance using the disease indices of all 44 hybrids. The alleles that are significant at $p \leq 0.05$ with absolute value of correlation coefficient >0.3 ($|r| > 0.3$) were considered associated with the resistance in present research to ensure a higher reliability. The results showed that three nuclear alleles (STI0002.108, STI0056.173, and STI0057.195) had negative correlation with increase of the disease index (Table 5). STI0002 and STI0057 locate on chromosome 9 and STI0056 locates on chromosome 2 (Table 5), suggesting that bacterial wilt resistance of wild fusion parent may be not controlled by a single gene, these three SSR alleles may be potentially linked to the genes on the two chromosomes that contribute to bacterial wilt resistance in the hybrids.

Discussion

In the present research, a total 317 nuclear alleles detected by 108 SSR markers, 5 cytoplasmic alleles detected by 4 mtDNA markers, and 1 cpDNA marker were investigated to clarify the genomic components of 44 somatic hybrids previously obtained by protoplast fusion between *S. tuberosum* and *S. chacoense*. The 268 polymorphic alleles are the sum of 159 from the cultivated type $3^{\#}$ and 109 from the wild type C9701 and were distributed over all the 12 chromosomes (Table 1), suggesting that these

Table 3 Cytoplasmic genomic components of the somatic hybrids

	mtDNA pattern		cpDNA pattern		
	Primer8/10/15/16		NTCP-9		
Genotypes	2009	2011	2003	2005	2010
3#	00 cc	00 cc	c	c	c
C9701	ww00	ww00	w	w	w
3C1-1	wwcc	wwcc	cw	c	c
3C1-2	wwcc	wwcc	w	w	cw
3C1-3	wwcc	wwcc	c	c	c
3C1-4	wwcc	wwcc	cw	w	w
3C1-5	0wcc	0wcc	c	c	c
3C2-1	wwcc	wwcc	c	c	c
3C2-3	0wcc	0wcc	cw	c	c
3C3-1	0wcc	0wcc	nt	nt	nt
3C3-3	ww00	ww00	w	w	w
3C3-4	nt	wwcc	cw	w	nt
3C3-5	0wcc	0wcc	w	w	w
3C3-6	0wcc	0wcc	w	w	w
3C3-7	0wcc	0wcc	w	w	w
3C3-8	0wcc	0wcc	w	w	w
3C6-2	wwcc	wwcc	w	nt	w
3C7-1	wwcc	wwcc	w	w	w
3C8-1	ww0c	w0cc	w	w	w
3C8-2	ww00	0w00	w	w	w
3C8-3	wwcc	0w00	w	c	w
3C8-4	ww00	ww0c	w	w	w
3C10-1	wwcc	wwcc	w	w	w
3C10-2	0wcc	00 cc	c	c	c
3C12-1	wwcc	wwcc	c	c	c
3C12-2	0w0c	0wcc	c	c	c
3C14-1	0wcc	0wc0	w	w	cw
3C15-1	wwcc	wwcc	c	c	c
3C18-1	wwcc	wwcc	c	c	c
3C19-1	0wcc	0wcc	c	c	c
3C21-1	wwcc	wwcc	c	w	w
3C25-3	wwcc	wwcc	c	c	c
3C27-1	wwcc	wwcc	c	c	w
3C28-1	wwcc	wwcc	c	c	c
3C29-2	0wcc	0wcc	w	w	w
3C30-1	0wcc	0wcc	c	c	c
3C30-2	0wcc	0wcc	c	c	c
3C31-1	0wcc	0wcc	w	w	w
3C33-1	wwcc	wwcc	w	w	w
3C33-2	0wcc	0wcc	c	c	c
3C34-1	ww00	ww00	w	w	w
3C35-1	wwcc	wwcc	c	c	c
3C35-2	nt	0wcc	c	c	nt
3C35-3	0wcc	wwcc	c	nt	c
3C36-1	0wcc	0wcc	w	w	w
3C39-1	wwcc	wwcc	nt	nt	nt

c showed the 3# cp/mtDNA pattern, *w* showed the C9701cp/mtDNA pattern, *0* showed no amplification product, *nt* not tested

polymorphic alleles could indicate the nuclear genome components of the somatic hybrids. Although only five cytoplasmic markers showed polymorphism between the fusion parents, they are specific to mitochondria and chloroplast organelles and could be used for cytoplasmic genome identification. Similar studies have been reported by Lovene et al. (2007) who used two cpDNA and two mtDNA polymorphism markers to detect the cytoplasmic genome composition of the somatic hybrids between *S. bulbocastanum* and *S. tuberosum*, and Patel et al. (2011) who tested the cytoplasmic components of tobacco somatic hybrids with one cpDNA and one mtDNA specific primer.

The polymorphic alleles of both fusion parents could be detected in 44 of the somatic hybrids (Table 1). This suggested that the symmetric protoplast fusion carried out previously ensured efficient genome variations. Our results further demonstrated that individual somatic hybrids showed distinct nuclear genomic components, since 138–158 alleles of the cultivated parent and 85–102 alleles of the wild parent could be detected in different hybrids. Multiple fusions, fusion of protoplasts at different phases of the cell cycle, and protoclonal variation (Lovene et al. 2007) and chromosome elimination (Pijnacker et al. 1989) could explain this phenomenon. For example, during callus formation and plant regeneration, the parental chromosomes might exclude or balance each other, and this could induce some deletion or rearrangement of chromosomes or chromosomal fragments (Babiychuk et al. 1992; Boltowicz et al. 2005). Two novel nuclear alleles were detected by the SSR marker STM2022 in 43 of the 44 somatic hybrids implying possible integration of parental DNAs as found in similar work by Bidani et al. (2007).

The results showed that the parental mitochondria genome DNA tended to rearrange in most somatic hybrids, whereas the chloroplast genome DNA seemed to be more conservative with 78.6 % of the somatic hybrids (33 out of 44) having the chloroplast type of one of the fusion parents (Table 3). This suggests that there may be some form of incompatibility between the parental organelles. The higher frequency of mtDNA recombination than cpDNA in the somatic hybrids may due to the structure of mitochondria which contains some master circles and subgenomic circles as indicated in *Nicotiana tabacum*, or a large

Table 4 The disease index, relative disease index and resistance level of the somatic hybrids and the fusion parents to bacterial wilt

Genotypes	Disease index[a] (mean ± SD)	Relative disease index (%)	Resistance level[b]
C9701	0.286 ± 0.025^{A}	71.0	R
3C3-4	0.301 ± 0.086^{A}	69.5	MR
3C1-1	0.326 ± 0.151^{A}	66.9	MR
3C25-3	0.344 ± 0.098^{A}	65.1	MR
3C1-2	0.420 ± 0.396^{AB}	57.4	MR
3C8-2	0.510 ± 0.160^{ABC}	48.3	MR
3C28-1	0.537 ± 0.154^{ABCD}	45.5	MR
3C29-2	0.557 ± 0.197^{ABCDE}	43.5	MR
3C8-3	0.667 ± 0.290^{BCDEF}	32.4	MS
3C7-1	0.694 ± 0.056^{BCDEF}	29.6	MS
3C19-1	0.741 ± 0.0^{CDEF}	24.8	MS
3C1-3	0.765 ± 0.116^{CDEF}	22.4	MS
3C8-1	0.785 ± 0.156^{CDEF}	20.4	MS
3C27-1	0.787 ± 0.082^{CDEF}	20.2	MS
3C8-4	0.802 ± 0.294^{CDEF}	18.7	S
3C1-4	0.817 ± 0.115^{CDEF}	17.1	S
3C15-1	0.825 ± 0.233^{CDEF}	16.3	S
3C18-1	0.825 ± 0.037^{CDEF}	16.3	S
3C30-1	0.829 ± 0.035^{CDEF}	15.9	S
3C33-1	0.840 ± 0.278^{CDEF}	14.8	S
3C3-7	0.864 ± 0.180^{DEF}	12.4	S
3C35-3	0.877 ± 0.156^{EF}	11.1	S
3C14-1	0.898 ± 0.0^{F}	8.9	S
3C10-1	0.903 ± 0.149^{F}	8.4	S
3C10-2	0.904 ± 0.030^{F}	8.3	S
3C30-2	0.905 ± 0.190^{F}	8.2	S
3C12-2	0.912 ± 0.005^{F}	7.5	S
3C36-1	0.919 ± 0.094^{F}	6.8	S
3C31-1	0.925 ± 0.090^{F}	6.2	S
3C12-1	0.943 ± 0.011^{F}	4.4	S
3C3-6	0.949 ± 0.084^{F}	3.8	S
3C2-3	0.963 ± 0.0^{F}	2.3	S
3C3-3	0.972 ± 0.037^{F}	1.4	S
3C33-2	0.972 ± 0.019^{F}	1.4	S
3C21-1	1.0 ± 0.0^{F}	1.4	S
3C3-1	1.0 ± 0.0^{F}	1.4	S
3C34-1	1.0 ± 0.0^{F}	1.4	S
3C39-1	1.0 ± 0.0^{F}	1.4	S
3C35-2	0.980 ± 0.040^{F}	0.6	S
3C35-1	0.981 ± 0.019^{F}	0.5	S
3C6-2	0.981 ± 0.0^{F}	0.5	S
3C1-5	0.986 ± 0.028^{F}	0.0	S
3C2-1	0.986 ± 0.012^{F}	0.0	S
3C3-5	0.991 ± 0.009^{F}	0.5	S
3C3-8	0.991 ± 0.009^{F}	0.5	S
3[#]	0.986 ± 0.028^{F}	0.0	S

[a] The same capital alphabet represents no significance at $P = 0.01$

[b] *R* resistant, *MR* medium resistant, *MS* medium susceptible, *S* susceptible

number of repeated sequences varied in size from 2,427 bp in rapeseed to 127,600 bp in rice (Sugiyama et al. 2005). The stability of parental cpDNA in the somatic hybrids might reflect the small genome size (155–156 kb) containing two invert repeat regions of 23–27 kb (Heinhorst et al. 1988) of potato chloroplasts or a difference in the rate

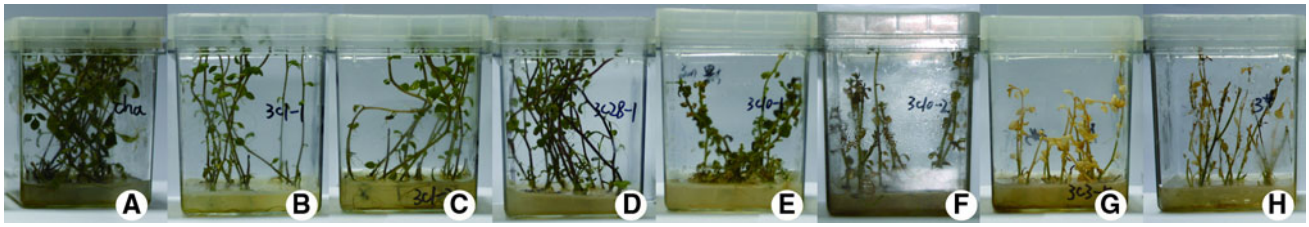

Fig. 3 Symptoms of the in vitro plants after inoculation. **a** C9701 (resistant fusion parent); **b–d** medium resistant somatic hybrids, **b** 3C1-1, **c** 3C1-2, **d** 3C28-1; **e–g** susceptible somatic hybrids, **e** 3C10-1, 3C10-2, **g** 3C3-5; **h** $3^{\#}$ (susceptible fusion parent)

Table 5 The nuclear SSR alleles which associated with disease index

Marker alleles	Chromosome location	Spearman correlation coefficient	*P* value
STI0002.108	9	−0.353	0.016
STI0056.173	2	−0.374	0.010
STI0057.195	9	−0.326	0.027

of organelle replication (Collonnier et al. 2001). Similar results have been reported by Cardi et al. (1999) and Bastia et al. (2000). Goremykin et al. (2008) also suggested that in higher plant, the mitochondrial structure changes rapidly and the rearrange ratio was much higher than chloroplast genome and animal mitochondria.

The disease resistance evaluation showed that the somatic hybridization could transfer bacterial wilt resistance from wild species *S. chacoense* into *S. tuberosum* (Table 4). In our followed-up research, three resistant somatic hybrids are fertile; one of them (3C28-1) was successfully backcrossed to the cultivated genotypes. The backcross progenies have been preliminarily tested for bacterial wilt resistance and some showed a similar resistance level to the original wild fusion parent (data not shown), suggesting potential values of the resistant somatic hybrids in potato breeding. Further analysis showed that three nuclear SSR alleles from the resistant wild parent associated with the resistance to bacterial wilt (Table 5), suggesting that the bacterial wilt resistance in potato may be not conferred by a single gene and these alleles are potentially linked to, or may be the resistance genes controlling the resistance. Our results may provide molecular evidence for the hypothesis that multigenes participate in potato resistance to bacterial wilt (Gao et al. 2000; Tung 1992). One of the resistant alleles, STI0056.192, has 58 % homology with the phosphoinositide-specific phospholipase C (PI-PLC) in tobacco. PI-PLC is considered as an important enzyme during the phosphoinositide signal route which plays a crucial role in the growth, development and response to the environmental stress, or pathogen attack in carrots (Kurosaki et al. 1987) and wheat (Melin et al. 1992). The functions of these alleles in potato resistance against bacterial wilt are worth further investigation.

Changes in the cytoplasmic genome components in subcultures of somatic hybrids observed in the present research have also been recognized previously. Jähne et al. (1991) found that in barley long-term culture causes deletions in plastid genome. Kawata et al. (1995) analyzed plastid DNA of rice regenerants maintained in vitro for several years and found a deletion that covered a large region of the genome, confirming that long-term culture can cause mutations at the plastid DNA level. The genetic background might also affect the genome stability of the hybrids.

In conclusion, the fusion that occurred in both nuclear and cytoplasmic genomes ensured that the somatic hybrids obtained desired traits from one of the fusion parents. The analysis of the genomic components revealed that three nuclear alleles were associated with potato resistance to bacterial wilt. Usage of the resistant somatic hybrids and selection for the resistant alleles may have potential to improve potato resistance against bacterial wilt.

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