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ORIGINAL PAPER

Nuclear DNA content and chromosome number in somatic hybrid allopolyploids of *Solanum*

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Abstract An attempt was made to change the proportion of the parental genomes in interspecific hybrids *Solanum nigrum* + *S. tuberosum* (*ngr* + *tbr*) by means of repeated protoplast fusion. In order to enlarge the potato input into the hybrid genome, the protoplasts of two *ngr* + *tbr* hybrids of different ploidy (7*x* and 8*x*) were fused with the protoplasts of two different diploid potato clones in three combinations. Protoclonal variability was studied in three populations of new *ngr* + *tbr* allopolyploids maintained in vitro. The absolute nuclear DNA content (2C) was measured using flow cytometry to estimate the ploidy of the hybrids. The ploidy level of the selected clones was verified by chromosome counts in root meristems. The newly synthesized allopolyploids (75 clones) showed only a small gain in nuclear DNA content above the mean value determined for the parents, instead of the expected addition of an entire diploid potato genome to the combined parental *ngr* + *tbr* genome. An increase in nuclear DNA was observed mostly in the clones having the 7*x* hybrid as a parent (75% of allopolyploids from two combinations). When the 8*x* hybrid was used as a parent, only two allopolyploids (5%) exhibited a significantly increased nuclear DNA content. The 8*x* level of *ngr* + *tbr* allopolyploids was shown to be stable and was only occasionally exceeded. Somatic hybrids *ngr* + *tbr* offer a model system for studying the molecular mechanism(s) and processes involved in stabilization and establishment of the synthetic *Solanum* allopolyploids.

Keywords Nuclear DNA content · Flow cytometry · Somatic hybridization · Synthetic *Solanum* allopolyploids · Chromosome number · Genome size

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Introduction

Potato is one of the major agricultural crops worldwide. The potato industry requires new cultivars with high productivity and disease resistance, especially to *Phytophthora infestans*, the most devastating pathogen of cultivated potato. For potato crop improvement, biotechnological methods are widely applied, including somatic hybridization technique, allowing the enrichment of potato gene pool through introgression of valuable genes from the remote wild species into cultivated potato (Song et al. 2003; Colton et al. 2006; Halterman et al. 2008).

Interspecific somatic hybrids of potato could be referred to as synthetic allopolyploids with combined genomes of cultivated potato and more or less closely related wild *Solanum* species. Such hybrids are often resistant to disease due to the presence of *R* (resistance) genes originating from the gene pool of wild species (Szczerbakowa et al. 2003). The resistant interspecific hybrids might be used for breeding resistant potato varieties. Successful introgression of resistance genes into breeding material demands knowledge on degree of incompatibility of the parental genomes and its consequences for the breeders. Although there are many natural allopolyploids of tuber-bearing potato, including such species as hexaploid *S. demissum* and tetraploid *S. hjertingii*, *S. papita*, and *S. stoloniferum* (Pendinen et al. 2008; Spooner et al. 2008), there is a lack

of information on the character of genome incompatibility in synthetic allopolyploids of *Solanum* in comparison with such taxons as *Arabidopsis*, *Brassica* or *Triticum*. According to McClintock (1984), the incompatibility of genomes in interspecific hybrids causes a genomic shock resulting in changes which are programmed responses to stress. An intriguing question concerns the mechanism(s) of stabilizing the newly formed allopolyploids, and the processes and factors involved therein. In allopolyploids, the genome destabilization and its rapid restructuring occur as a result of homeological recombination between highly repeatable DNA sequences and the loss of DNA fragments (Rogalska 2006). The allopolyploidy-induced, nonrandom DNA sequence elimination (the elimination of genome-specific and chromosome-specific sequences) during the stabilization of allopolyploids was shown by Osborn et al. (2003). In wheat, when the nuclear 2C DNA content of newly synthesized amphydiploids and their parents were compared, there was a significant reduction of genome size (Ozkan et al. 2003). A well-known event is chromosome loss in allopolyploids. For example, in intergeneric somatic hybrids between *Brassica oleracea* and *Matthiola incana*, both the nuclear DNA content and chromosome number were lower than the added values of the parental species indicating the partial chromosome loss (Sheng et al. 2008). Most of the chromosomes of *Arabidopsis thaliana* L. were eliminated in intergeneric somatic hybrids between *A. thaliana* and *Bupleurum scorzonerifolium* Willd. (Minqin et al. 2008). Another phenomenon caused by allopolyploidization is the silencing or reduced expression of duplicate genes (Pikaard 2001; Hegarty and Hiscock 2008). There are many evidences of gene silencing, i.e. the suppression of the expression of additional copies of genes in the allopolyploid nucleus (Adams and Wendel 2005).

The new genomic arrangements and cytogenetic behavior of synthetic allopolyploids is an important subject for investigation. A novel association of *S. nigrum* (*ngr*) and *S. tuberosum* (*tbr*) genomes within a single nucleus would also affect the organization of the DNA and gene expression. In this paper, we report the results of flow cytometric (FCM) and cytogenetic analyses of three populations of allopolyploids obtained by fusion between the protoplasts of *Solanum nigrum* + *S. tuberosum* (*ngr* + *tbr*, or NT) hybrids, described earlier (Szczerbakowa et al. 2003), and the protoplasts of diploid potato. The second fusion (re-fusion) of hybrid protoplasts with the potato ones was performed in order to enlarge the input of potato genome into the combined genome of *ngr* + *tbr* hybrids. The variability of nuclear DNA content of new (*ngr* + *tbr*) + *tbr* (or NTT) polyploids with increased genome size revealed by FCM analysis, was confirmed by karyotypic analysis of hybrid protoclones sustained in vitro. The octoploidy preference of the *ngr* + *tbr* hybrids was shown, as was the high frequency of aneuploids and mixoploids. Presented results demonstrated the genome size modifications and diversity in interspecific *ngr* + *tbr* hybrids obtained via protoplast fusion that led to polyploidization and generation of new synthetic *Solanum* allopolyploids combining both nuclear and cytoplasmic genomes of two distantly related species, *S. nigrum* and *S. tuberosum*.

Materials and methods

The parental clones

Two hybrids, NT81 ($2n = 8x = 96$) and NT109 ($2n = 7x = 84$), from the population of somatic hybrids between *S. nigrum* ($2n = 6x = 72$) and *S. tuberosum* clone ZEL-1136 ($2n = 2x = 24$) (Szczerbakowa et al. 2003), were used in re-fusion experiments with two potato diploids DG 81-68 ($2n = 2x = 24$) and DG $D_2$413 ($2n = 2x = 24$) (kindly provided by The Młochów Unit of IHAR) in order to increase the input of potato genome into the *ngr* + *tbr* allopolyploids. Somatic embryos were formed within 3–4 months after the fusion. One shoot was detached from each of the most rapidly regenerating calli and rooted, forming an individual protoclone. The protoclones were maintained on hormone-free ½ MS medium (Murashige and Skoog 1962) with 0.8% agar.

Protoplast isolation, fusion and regeneration

The applied technique of protoplast isolation and regeneration was performed as described previously (Szczerbakowa et al. 2000). Parental protoplasts in a 1:1 mixture at final density of 10^6 cm^{-3} were fused in a medium consisting of 25% PEG 6,000, 0.1 M $Ca(NO_3)_2$, 0.3 M mannitol and 10% DMSO, adjusted to pH 9.0. The numbers of plants regenerated from three fusion combinations are presented in Table 1.

Flow cytometry

Leaf samples were prepared as described by Sliwinska and Thiem (2007), using Galbraith's buffer (Galbraith et al. 1983) supplemented with propidium iodide (PI; 50 μg cm^{-3}), ribonuclease A (50 μg cm^{-3}) and 1% (w/v) polyvinylpyrrolidone (PVP-10). *Zea mays* (CE-777; 5.43 pg/2C; Lysák and Doležel 1998) was used as an internal standard. Fluorescence of PI was measured in 5,000–8,000 nuclei per sample using a Partec CCA (Partec GmbH, Münster, Germany) flow cytometer, and for histograms evaluation a DPAC v.2.2 computer program was applied. Nuclear DNA content was

Table 1 Efficiency of somatic hybridization in terms of the number of *ngr* + *tbr* allopolyploids with increased nuclear DNA content

Combination	Number of clones	Number of clones with increased nuclear DNA level	Mean DNA gain (pg)[a]
NT81 + *tbr* DG 81-68	40	2 (5%)	0.42
NT109 + *tbr* DG 81-68	23	17 (74%)	0.32
NT109 + *tbr* DG $D_2$413	14	11 (78%)	0.38

[a] Calculated in relation to the value 8.75 pg for NT81 and 7.60 pg for NT109 (see text for explanation)

calculated using the linear relationship between the ratio of the 2C peak positions of target plant/internal standard on the histogram of fluorescence intensities.

Chromosome count

The root tips (5–10 mm) of in vitro-cultured plants (8–10 plants per clone) were detached 6–9 days after the last passage, pretreated with 2 mM 8-hydroxyqinoline for 6 h, and fixed in Carnoy solution (ethanol:acetic acid, 3:1) for 48 h at room temperature. The fixed tissues were digested for 30 min at 37°C with the following solution: 1% cellulase Onozuka R-10, 1% cellulase from *Aspergillus niger*, 0.32 units/mg (Serva), 20% pectolyase, 0.70 units/mg (Serva), 1 mM EDTA, and 10 mM citric buffer, pH 4.8. The mitotic plates were examined in the best preparations from random root tips (4–12 preparations per clone). The root tip squashes were stained with DAPI and examined under UV with an inverted microscope Olympus IX-70 and filter cube U-MNU (360–370 nm excitation and 420 nm emission).

Results

The parental clones

The nuclear DNA histograms of the parental clones differing in ploidy are presented in Fig. 1. In the diploid potato clone DG 81-68, all the checked cells had 24

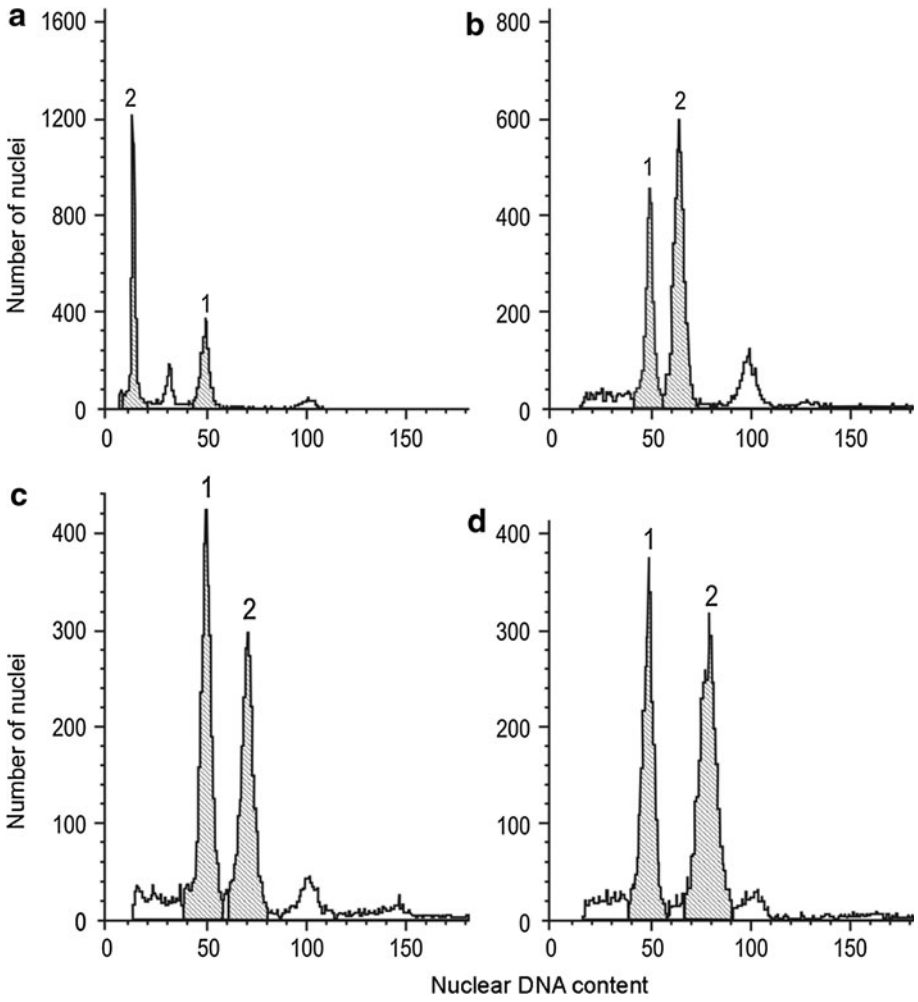

Fig. 1 DNA histograms of nuclear preparations from leaves of: **a** 2*x* *S. tuberosum* DG 81-68; **b** 6*x* *S. nigrum*; **c** 7*x* NT109; **d** 8*x* NT81. 1: 2C peak of internal standard *Zea mays* CE-777; 2: 2C peak of a specimen

chromosomes. The hybrid *ngr* + *tbr* NT81 was a typical octoploid with 96 chromosomes. The 2C DNA (C-value: DNA content of a holoploid genome with chromosome number *n*; Greilhuber et al. 2005) for the *ngr* + *tbr* octoploid clone NT81 was 8.75 ± 0.05 pg and was about 5% higher than the sum of the parental 2C values for *S. tuberosum* (1.56 pg) and *S. nigrum* (6.79 pg), i.e. 8.35 pg (Table 2). The cytogenetic analysis confirmed the presence of 96 chromosomes in metaphase plates of the NT81 clone. On the other hand, the hybrid *ngr* + *tbr* NT109 possessed only 84 chromosomes (7*x*) and 7.6 pg/2C (Table 3). Nevertheless, the 1Cx-value (DNA content of one non-replicated monoploid genome with chromosome number *x*; Greilhuber et al. 2005) of hybrids 8*x* and 7*x* was similar, 1.09 and 1.08 pg, respectively (Tables 2, 3, 4). Morphological traits of the parental hybrids were quite similar, despite the differences in ploidy. The hybrids' simple leaves were of the wild type, although wrinkled and slightly irregular. The 7*x* NT109 plants were usually taller than those of the 8*x* NT81 clone. Both clones set flowers in vitro, but did not form seeds, as did the parental self-compatible species *S. nigrum.*

Allopolyploids 8*x* NT81 + 2*x tbr* DG 81-68

Surprisingly, the protoplast fusion between 8*x* NT81 and 2*x* DG 81-68 yielded mostly octoploids (95%) and only two hyperoctoploids (5%; Table 5). Of forty NTT81 clones screened, the eight selected ones are presented in Table 2. Only one mixoploid was found (NTT81/25) and only two clones, NTT81/17 and NTT81/27, had a significantly increased DNA content (8.89 and 9.44 pg, respectively). For the remaining NTT81 clones, the mean DNA content (8.70 pg) and 1Cx DNA (1.08 pg) were similar to those of the parental hybrid NT81 (8.75 pg and 1.09 pg, respectively). The results confirmed the stability of the *ngr* + *tbr* octoploids during the repeated protoplast isolation and fusion procedures followed by the process of regeneration through the callus stage.

Allopolyploids 7*x* NT109 + 2*x tbr* DG 81-68

The fusion between the protoplasts of 7*x* NT109 and 2*x* DG 81-68 was effective and yielded 74% regenerants with an increased nuclear DNA content (Table 3). Their ploidy

Table 2 Nuclear DNA content in the allopolyploids from the combination 8*x* NT81 + 2*x tbr* DG 81-68

Genotype	2C DNA (pg ± SE) (number of plants analyzed)	Gain or loss of DNA (pg)	1Cx (pg)
S. nigrum L. (6*x*)	6.79 ± 0.10 (7)		1.13
tbr DG 81-68 (2*x*)	1.56 ± 0.02 (7)		0.78
(*ngr* + *tbr*) NT81 (8*x*)[a]	8.75 ± 0.05 (8)		1.09
NTT81/3	8.60 ± 0.04 (3)	−0.15	1.08
NTT81/6	8.45 ± 0.12 (2)	−0.30	1.06
NTT81/17	8.89 ± 0.08 (5)	0.14	1.11
NTT81/27	9.44 ± 0.08 (6)	0.69	1.18
NTT81/29	8.82 ± 0.06 (8)	0.07	1.10
NTT81/30	8.81 ± 0.14 (4)	0.06	1.10
NTT81/33	8.75 ± 0.07 (7)	0.00	1.09
NTT81/39	8.76 ± 0.04 (5)	0.01	1.09

[a] Expected values: 2C = 8.35 pg (*ngr* + *tbr*), 1Cx = 1.04 pg

Table 3 Nuclear DNA content in selected allopolyploids from the combination 7*x* NT109 + 2*x tbr* DG 81-68

Genotype	2C DNA (pg ± SE) (number of plants analyzed)	Gain or loss of DNA (pg)	1Cx (pg)
S. nigrum (6*x*)	6.79 ± 0.10 (7)		1.13
tbr DG 81-68 (2*x*)	1.56 ± 0.02 (7)		0.78
(*ngr* + *tbr*) NT109 (7*x*)	7.60 ± 0.05 (5)		1.08
NTT109/2	6.61 ± 0.01 (3)	−0.99	0.87
NTT109/4	7.79 ± 0.08 (5)	0.19	1.11
NTT109/6	8.22 ± 0.05 (5)	0.62	1.17
NTT109/12	7.81 ± 0.08 (3)	0.21	1.11
NTT109/17	8.24 ± 0.07 (4)	0.64	1.18
NTT109/21	7.93 ± 0.09 (3)	0.33	1.13
NTT109/31	7.98 ± 0.04 (2)	0.38	1.14

Table 4 Nuclear DNA content in allopolyploids from the combination NT109 + *tbr* DG $D_2$413

Genotype	2C DNA (pg ± SE) (number of plants analyzed)	DNA gain (pg)	1Cx (pg)
S. nigrum (6*x*)	6.79 ± 0.01 (7)		1.13
tbr DG $D_2$413 (2*x*)	1.66 ± 0.01 (2)		0.78
(*ngr* + *tbr*) NT109 (7*x*)	7.60 ± 0.05 (5)		1.08
NTT109/A	7.95 ± 0.14 (3)	0.35	1.13
NTT109/D	7.96 ± 0.08 (7)	0.36	1.14
NTT109/F	8.19 ± 0.09 (6)	0.59	1.17
NTT109/G	7.81 ± 0.11 (3)	0.21	1.11
NTT109/J	7.82 ± 0.09 (3)	0.22	1.12
NTT109/L	7.97 ± 0.12 (3)	0.37	1.14
NTT109/M	8.17 ± 0.05 (7)	0.57	1.17
NTT109/O	7.92 ± 0.11 (3)	0.32	1.13
NTT109/P	7.99 ± 0.09 (3)	0.39	1.14
NTT109/R	7.94 ± 0.11 (3)	0.34	1.13

level could be characterized as 8*x*- (hypooctoploid). A maximal gain in nuclear DNA content (an increase in comparison with the DNA content of the parental clone NT109) was 0.64 pg in the clone NTT109/17. Surprisingly, there was a casual loss of DNA in the clone NTT109/2. However, karyotype screening as well as FCM revealed that a high proportion of the NTT109/2 plants had variable ploidy (Table 5, Fig. 2). Out of 51 plants screened for this combination, 16 plants (31%) were mixoploid; according to FCM histograms, in some of the NTT109 clones, besides cells with DNA content of about 8 pg (Table 3), there was a considerable proportion of cells with a lower DNA content of 4.2–6.9 pg (Fig. 2). They corresponded to the cells with 4*x*–6*x* chromosomes detected microscopically. To detect the peak corresponding to the nuclei with 5*x* chromosomes, which was close to that of *Z. mays* used as an internal standard, additional samples without internal standard were also run (Fig. 2B).

Allopolyploids 7*x* NT109 + 2*x* *tbr* DG $D_2$413

When another diploid potato clone, DG $D_2$413, was used instead of clone DG 81-68 in combination with NT109, the fusion was also effective and 78% of the regenerants showed a significant gain in nuclear DNA content (Table 1). This gain amounted to 0.6 pg in two regenerants, NTT109/F and NTT109/M (Table 4), while the mean gain was calculated as 0.38 pg (Table 1). The ploidy level of the hybrids from this combination could be characterized as 8*x*- (hypooctoploid).

Interestingly, some leaves of the same hybrid clone were mixoploid (Fig. 2). In the case of the NT109 hybrid and its re-fusion regenerants, both mixoploid and euploid plants were found within the same clones. The 2C DNA value determined for this heptoploid was 7.60 pg. After re-fusion, a significant increase in nuclear DNA content was observed in 74% of the NTT109 clones. Their ploidy in most cases was 8*x*-. Although the re-fusion of NT109 hybrid with diploid potato (both DG 81-68 and DG $D_2$413) was successful, mixoploidy disqualifies most of the NTT109 allopolyploids for practical use.

Chromosome count

The FCM data were supplemented by karyotypic analysis of selected hybrids. The cytogenetic analysis confirmed an increased number of chromosomes in the refusants with an increased nuclear DNA content in comparison with the parental clones, NT81 and NT109 (Table 5). For example, 68% of nuclei of the hybrid NTT81/27 contained 100–120 chromosomes (Fig. 3A), and 70% of nuclei of the hybrid NTT109/M contained 85–90 chromosomes. In some metaphase plates of clone NTT109/2, the chromosome number was highly polyploid (Fig. 3B), while in others it was 67–79 and even lower (Table 5). The variable level of ploidy was characteristic for the hybrids from all three combinations, as judged by karyotypes in root meristem cells (Table 5, Fig. 3).

Discussion

The nuclear genome size of the parental species used in the synthesis of the allopolyploids, *S. tuberosum* (*tbr*) and *S. nigrum* (*ngr*), as well as their somatic hybrids obtained via protoplast fusion has been measured using flow cytometry. The absolute DNA content in three potato diploids was 0.78 pg/1Cx, while in *S. nigrum* it was about 45% higher and amounted to 1.13 pg/1Cx, thus the monoploid genome of *ngr* was larger than that of *tbr*. The absolute DNA

Table 5 Chromosome number in root meristem cells of the selected *ngr* + *tbr* allopolyploids

Hybrid clone (total number of mitotic plates examined)	Chromosome number	Number of mitotic plates	Ploidy (number of chromosomes)
NT81	72	2	6*x* (72)
(14)	96	12 (86%[a])	8*x* (96)
NT109	70–75	6 (29%)	6*x* ± (< 72 <)
(21)	76–86	14 (67%)	7*x* ± (< 84 <)
	87–96	1	8*x* − (≤ 96)
NTT81/17	38–52	5	4*x* ± (< 48 >)
(50)	62–73	5	6*x* ± (< 72 >)
	80–98	10 (50%)	8*x* ± (< 96 >)
NTT81/27	24	1	2*x* (24)
(20)	48	5	4*x* (48)
	50–72	10	≤6*x* (≤ 72)
	100–125	34 (68%)	<10*x* < (< 120 <)
NTT109/2	41	1	4*x* − (< 48)
(27)	62	1	5*x* + (> 60)
	67–79	9	6*x* ± (< 72 <)
	91–109	5	8*x* ± (< 96 <)
	114–123	3	10*x* ± (< 120 <)
	147	1	12*x* + (> 144)
	168–173	4	14*x* + (≥ 168)
	222–234	3	20*x* − (< 240)
NTT109/17	80–86	8 (36%)	7*x* ± (< 84 <)
(22)	87–90	14 (64%)	8*x* − (< 96)
NTT109/M	24	2	2*x* (24)
(30)	48	1	4*x* (48)
	72	2	6*x* (72)
	80–84	4 (13%)	7*x* − (≤ 84)
	85–90	21 (70%)	8*x* − (< 96)

[a] Percentage of the total number of mitotic plates examined per clone

content of the parental species was stable during a year of in vitro propagation, when the measurements by FCM were performed three times in several repeats at several months' intervals. The 2C-values for the diploid *S. tuberosum* clones examined here were close to those presented in earlier publications (1.77, 1.62 and 1.38 pg; Marie and Brown 1993, Valkonen et al. 1994, Maciejewska et al. 1999, respectively), However, the 2C value for *S. nigrum* reported before (Maciejewska et al. 1999), when trout erythrocytes were used as an external standard and DNA was stained with DAPI, was much lower (4.31 pg) than that obtained here (6.79 pg). However, the use of a base (AT) specific dye (DAPI) and an animal standard with different base composition could generate errors in plant genome size determination (Doležel 1991; Marie and Brown 1993). The somatic hybrids generated from widely divergent species, *S. nigrum* and *S. tuberosum,* were expected to have six complete sets of *S. nigrum* chromosomes ($2n = 6x = 72$) and two sets of *S. tuberosum* chromosomes ($2n = 2x = 24$) in their nuclei, although, in practice, many of the *ngr* + *tbr* hybrids were aneuploid or mixoploid (Szczerbakowa et al. 2003).

Flow cytometry (FCM) is widely used to study the genome size and stability in different plant materials cultured in vitro (e.g. Clarindo et al. 2008; Makowczynska et al. 2008; Mallón et al. 2010). FCM is also very helpful in the detection of variation in ploidy status among genotypes of the same species, e.g. *Brassica napus* (Takahira et al. 2011), as well as in interspecific hybrids (Tiwari et al. 2010). The variability in nuclear genome size of the *ngr* + *tbr* allopolyploids maintained in vitro probably reflected somaclonal variation occurring during protoplast regeneration through the callus dedifferentiating stage. According to Aversano et al. (2009), who studied the variability of nuclear and cytoplasmic DNA level in *Solanum* genotypes with a different genetic background and ploidy (wild species and hybrids, including *S. nigrum* and a somatic hybrid 4*x* S. *tuberosum* + 2*x* *S. bulbocastanum*), the integrity of the nuclear genome of the in vitro regenerants depended on the genotype. The nuclear genomic variation was found to be rather low in *S. nigrum* regenerants as judged by profiles of the polymorphic inter-

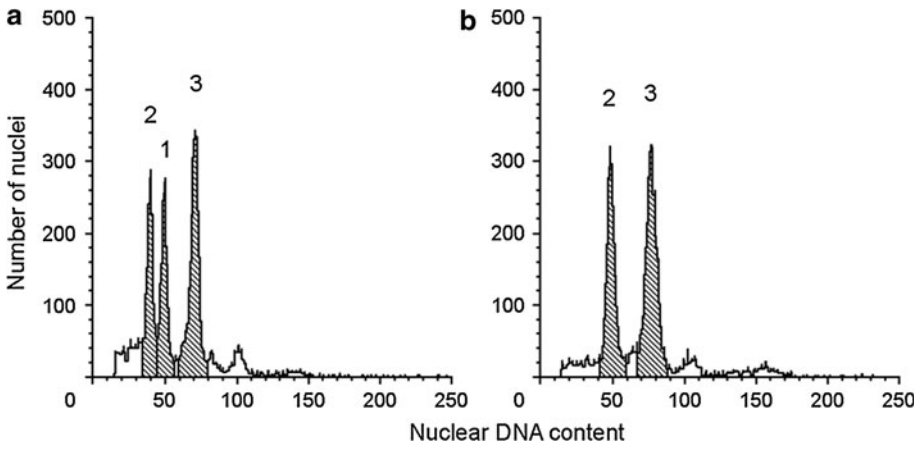

Fig. 2 DNA histograms of nuclear preparations from leaves of mixoploids. **a** NTT109/3 with internal standard *Zea mays* CE-777 (peak 1: nuclei with DNA content 5.43 pg; peak 2: nuclei with DNA content 4.34 pg; peak 3: nuclei with DNA content 7.71 pg); **b** NTT109/F without internal standard (peak 2: nuclei with DNA content about 5 pg; peak 3: nuclei with DNA content about 8 pg)

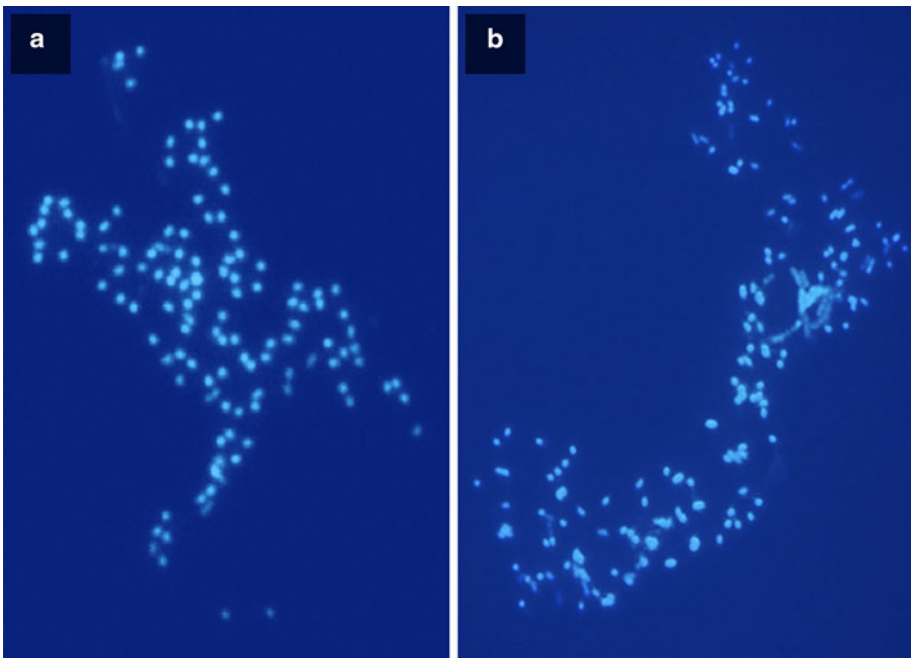

Fig. 3 The metaphase chromosomes in root meristem cells of the selected *ngr* + *tbr* allopolyploids. **a** NTT81/27 ($2n = 110$); **b** NTT109/2 ($2n = 170$)

simple sequence repeats (ISSR) markers. In other genotypes analyzed, the elimination of parental ISSR fragments occurred suggesting large rearrangements of nuclear genomes under in vitro culture conditions. In our studies, the tissue-culture associated alterations in the ploidy level detected by FCM in *ngr* + *tbr* regenerants, had no substantial effect on their morphology or on resistance to *P. infestans* (R. Lebecka, personal communication).

Our attempt to change the proportion of the parental genomes in interspecific hybrids between *Solanum nigrum* and *S. tuberosum* by means of repeated protoplast fusion (re-fusion) revealed the genome stability of the *ngr* + *tbr* octoploids. An attempt to increase the hybrid genome size above the initial octoploid level failed in most of the generated clones. On the other hand, it was possible to "complete" the *ngr* + *tbr* allopolyploid genome to the 8*x* level with potato DNA by protoplast re-fusion. The mean significant gain in nuclear DNA content (0.38 pg) corresponded to at least six added potato chromosomes and was confirmed by the higher chromosome number in the examined allopolyploid clones. The currently undertaken GISH analysis will allow verifying the potato origin of the additional chromosomes. However, in populations of such "completed" *ngr* + *tbr* allooctoploids, the protoclonal variability was high with considerable proportion of aneuploids, with DNA content both lower and higher than that of the euploids. Additionally, mixoploidy was observed in leaves of the individual plants of the same hybrid clone. Thus, the screened hybrid leaf tissue was apparently non-uniform in terms of ploidy. This phenomenon was confirmed by karyotypic analysis of the hybrid root meristem, where cells with different chromosome numbers were detected (see e.g. NTT109/2 in Table 5). Aneuploidy in the *ngr* + *tbr* hybrids had an impact on growth and development of the hybrid plants in vitro (stunted growth, leaf deformations). The stable *ngr* + *tbr* octoploids could be further used for identification of the origin of their chromosomes by molecular cytogenetic methods.

Combining of foreign genomes through protoplast fusion and heterokarion formation can result in spatial isolation and independent functioning of the parental genomes that lead to the mixoploidy of hybrid plant tissues. Since each parental species donates both the nucleus and the cytoplasm, genome readjustment is required for functioning in the new environment (Riddle and Birchler 2003). The adverse interactions between nuclear and cytoplasmic genomes result in sterility of the remote hybrids. Certain changes must occur in the nuclear genomes for restoration of fertility and nucleo-cytoplasm compatibility (Rieseberg 2001). It can also be assumed that a significant divergence between parental *ngr* and *tbr* genomes requires considerable chromosome rearrangements and intergenomic chromosome translocations for harmonious behavior and activity of the different constituent genomes. It is hard to predict the extent of genome readjustments at chromosome level caused by combination of divergent genomes of *S. nigrum* and *S. tuberosum* in newly formed polyploids. Consequently, somatic hybrids *ngr* + *tbr* offer a model system for studying the molecular mechanism(s) and processes involved in stabilization and establishment of synthetic *Solanum* allopolyploids.

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