Journal of Plant Research
https://doi.org/10.1007/s10265-021-01354-9

REGULAR PAPER – GENETICS/DEVELOPMENTAL BIOLOGY

Hybridization and polyploidization effects on LTR-retrotransposon activation in potato genome

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Received: 6 June 2021 / Accepted: 13 October 2021

Abstract
Hybridization and polyploidization are major forces in plant evolution and potatoes are not an exception. It is proposed that the proliferation of Long Terminal Repeat-retrotransposons (LTR-RT) is related to genome reorganization caused by hybridization and/or polyploidization. The main purpose of the present work was to evaluate the effect of interspecific hybridization and polyploidization on the activation of LTR-RT. We evaluated the proliferation of putative active LTR-RT in a diploid hybrid between the cultivated potato *Solanum tuberosum* and the wild diploid potato species *S. kurtzianum*, allotetraploid lines derived from this interspecific hybrid and *S. kurtzianum* autotetraploid lines (ktz-autotetraploid) using the S-SAP (sequence-specific amplified polymorphism) technique and normalized copy number determination by qPCR. Twenty-nine LTR-RT copies were activated in the hybrid and present in the allotetraploid lines. Major LTR-RT activity was detected in Copia-27, Copia-12, Copia-14 and, Gypsy-22. According to our results, LTR-RT copies were activated principally in the hybrid, there was no activation in allotetraploid lines and only one copy was activated in the autotetraploid.

Keywords Allotetraploid · Autotetraploid · Hybridization · Long terminal repeat retrotransposons · *Solanum kurtzianum* · *Solanum tuberosum*

Introduction

Along with polyploidization, transposable element (TE) amplification is considered the main mechanism by which plant genome sizes increase and, more broadly, evolve (Wendel et al. 2016). In fact, these two mechanisms seem to be related, reinforcing their potential to drive the evolution of plant genomes (Vicient and Casacuberta 2017). Polyploidization, especially allopolyploidization, is usually associated with rapid structural and functional alterations of genomes (Leitch and Leitch 2008), mainly in the repetitive components (Comai et al. 2003). Allopolyploidization is recognized as a major mechanism of adaptation and speciation in plants (Ramsey and Schemske 1998). Polyploidization has generally been assumed to induce a burst of transposition that could be related to specific elements (Comai et al. 2000). Besides, TE also appears to be associated with major structural changes such as recombination-driven sequence loss (Parisod et al. 2010).

TE expression and mobility seem to respond to specific stimuli, such as biotic and abiotic stresses like salt (Naito et al. 2009; Woodrow et al. 2010), wounding (Mhiri et al. 1997), temperature (Ishiguro et al. 2014; Ito et al. 2011; Ivashuta et al. 2002; Naito et al. 2009) and pathogens (Anca et al. 2014; Buchmann et al. 2009; Grandbastien et al. 2005). Hybridization and polyploidization are an example of "genome shock", a term proposed by McClintock (1984). As a response to stress conditions, genome shocks could cause the relaxation of gene expression, including TE activation, this process could be accompanied by rapid genetic and epigenetic changes, i.e. chromosomal rearrangements, disruption, loss of segments, alteration of DNA methylation,

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Published online: 21 October 2021

histone modifications and changes in small RNA expression. These changes may contribute to the stabilization of new species (De Storme and Mason 2014; Madlung et al. 2005; Tayalé and Parisod 2013).

The cultivated potato, *Solanum tuberosum* L., is the fourth most important crop in the world after maize, wheat, and rice (Devaux et al. 2014; FAOSTAT 2018). The tuber-bearing species of *Solanum* constitute an euploid series ranging from diploid (2n = 2x = 24) to hexaploid (2n = 6x = 72), hybridization and polyploidization are common events in this group (Masuelli et al. 2009). Following the genome sequencing of *S. tuberosum* group Phureja (Consortium 2011), bioinformatic analyses carried out to characterize the repetitive DNA content in the genome revealed that between 49 and 60% of the genome corresponds to repetitive sequences. In addition, the majority of retrotransposons detected belong to LTR-Gypsy and LTR-Copia retrotransposon superfamilies (Chandra Bhan Yadav 2013; Mehra et al. 2015; Zavallo et al. 2020). Deeper in silico analyses showed that only between 20 and 30% of total TE corresponds to previous known transposons (Mehra et al. 2015; Zavallo et al. 2020), with LTR-RTs being the most abundant, representing more than 80% of the total TE.

Particularly in tuber-bearing *Solanum* species, little is known about the activation of transposons due to polyploidization and hybridization. The effect of hybridization on the activity of retrotransposons Tto1 and Tnt1 was previously analysed in interspecific hybrids of *S. kurtzianum* Bitter & Wittm. and *S. microdontum* Bitter and in intraspecific crosses (Paz et al. 2015). Both retrotransposons were mobilized and, at the epigenetic level, demethylation occurred in the vicinity of Tnt1 and Tto1 in the hybrids compared with the parental genotypes (Paz et al. 2015). Epigenetic changes could influence both gene expression and transposon activation (Parisod et al. 2010; Shapiro 2014). In previous work, we studied the variability induced by whole-genome duplication in potato auto- and allotetraploids obtained through chromosomal duplication of a *S. kurtzianum* (ktz) genotype (autopolyploid model) and chromosomal duplication of a diploid interspecific hybrid between *S. tuberosum* (tbr) and ktz (allopolyploid model), respectively. Polyploid lines from both models showed a tendency towards increased vigour in phenotypic traits compared with their diploid parents (Marfil et al. 2018). While AFLP analysis showed no polymorphism between parental diploid and derived polyploids, DNA methylation assays indicate that polyploidization alters the epigenetic patterns in potato (Marfil et al. 2018). Therefore, the objective of this study was to investigate the effect of interspecific hybridization and whole-genome duplication on LTR-RT proliferation. We wanted to know whether hybridization together or separately with whole-genome duplication, induces LTR-RT activation. Taking advantage of available sequencing data, we identified specific LTR-RT and evaluated LTR-RT activation in the diploid interspecific hybrid between tbr and ktz, the ktz-autotetraploid, and the allotetraploid lines.

Materials and methods

Plant material and DNA extraction

We established three models to evaluate the activation of specific LTR-RT in potato: (i) hybridization model, (ii) allopolyploid model, and (iii) autopolyploid model. The parental species tbr and ktz, the interspecific diploid hybrid (2xPIH), four allotetraploid lines (4xAL1, 4xAL2, 4xAL3 and 4xAL4), and three ktz-autotetraploid lines (4xAuL1, 4xAuL2 and 4xAuL3) were analysed (Fig. 1a). Briefly, 2xPIH was obtained by embryo rescue from crosses between a dihaploid clone of tbr (2n = 2x = 24) and the genotype 2xPL of ktz (2n = 2x = 24) (Marfil et al. 2006). The allo- and ktz-autotetraploid lines were generated by in vitro treatment with colchicine of 2xPIH hybrid and with oryzalin of 2xPL, respectively (Marfil et al. 2018). For each analysed line, three biological replicates were clonally propagated by tubers during four generations under uniform conditions in an insect-proof greenhouse. All analyses were carried out in the fifth generation of clonal propagation. Genomic DNA was extracted from leaves according to Marfil et al. (2018) and DNA integrity was assessed by a 0.8% agarose gel. After spectrophotometric quantification (GeneQuant RNA/DNA Calculator, Pharmacia Biotech, England), DNA was diluted down to 50 ng μL^{-1} for downstream use in restriction and PCR protocols.

Identification and characterization of putative active LTR-Retroelements (LTR-RT) in *S. tuberosum* genome

Retrotransposon complete sequences present in the *S. tuberosum* reference genome (SolTub_3.0 NCBI assembly name and GCF_000226075.1 NCBI assembly accession) were obtained from the database REPBASE of the Genetic Information Research Institute (GIRI) (Jurka et al. 2005) and from the list of Zavallo et al. (2020). Putative LTR-RTs were subsequently analysed by searching for conserved domains in BLASTP and BLASTX (Altschul et al. 1990). Protein sequences were aligned and edited with Bioedit (Hall 1999) and ClustalX software (Larkin et al. 2007). For phylogenetic analysis, the most appropriate model of amino acid evolution for this region was inferred using MEGA (Kumar et al. 2018), WAG + G for Gypsy alignment and, rtREV + G for Copia alignment. Phylogenetic reconstruction by Maximum likelihood (ML) was estimated using the previously defined evolutionary

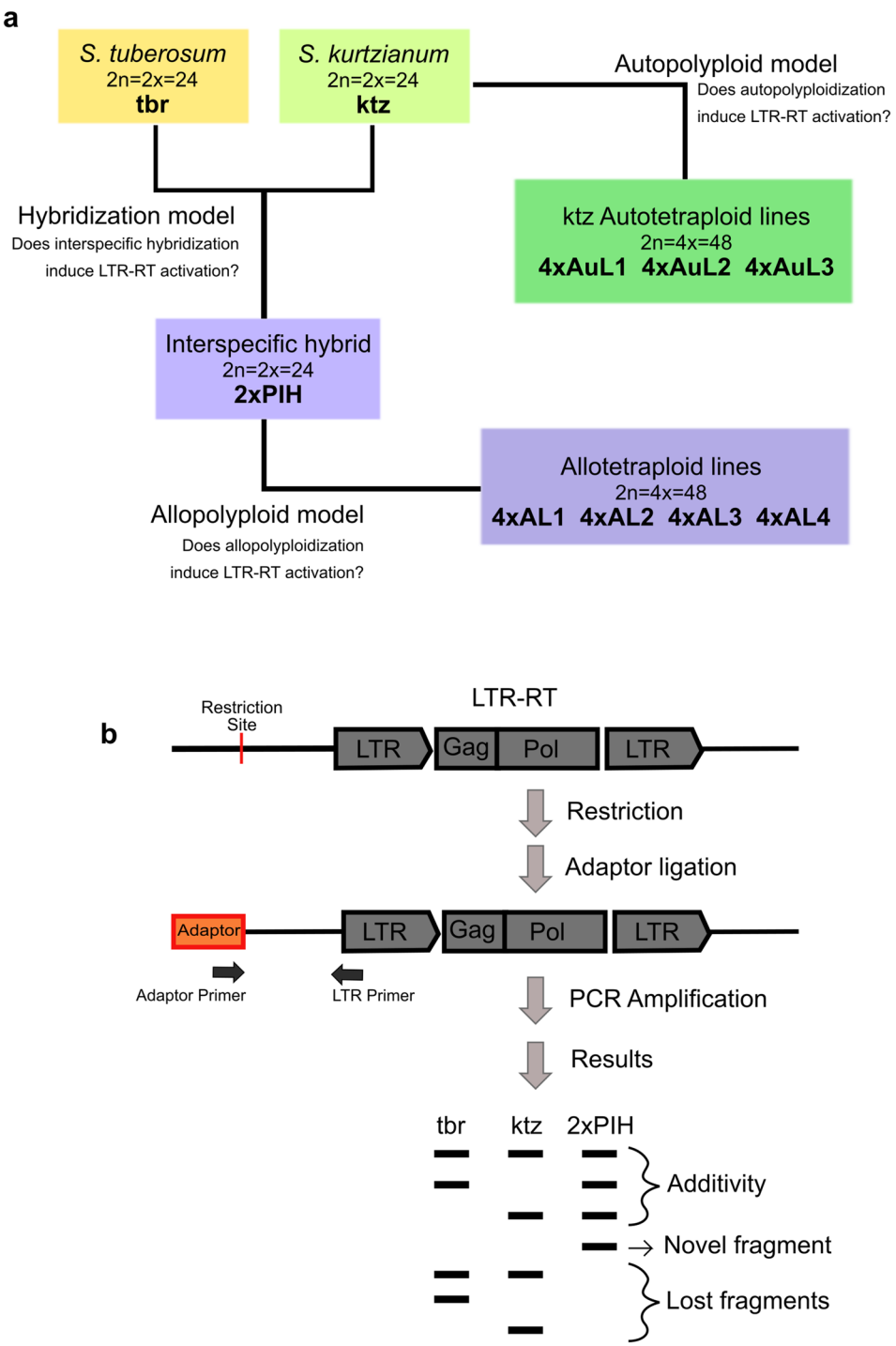

Fig. 1 Experimental models and molecular tools used to study the mobility of retrotransposon in potato. **a** Experimental models for studying LTR-RT activation induced by hybridization and polyploidization. Hybridization model, the interspecific hybrid (2xPIH) was obtained by a controlled sexual cross between the parental species *Solanum tuberosum* (tbr) and *Solanum kurtzianum* (ktz). Autopolyploid model, ktz-autotetraploid lines were obtained by chemical treatment of *S. kurtzianum*. Allopolyploid model, allotetraploid lines were obtained by chemical treatment of 2xPIH. **b** Determination of TE activation by Sequence-specific amplification polymorphisms technique (S-SAP). The final amplification involves two primers, one specific to the LTR-RT and the other to the adapter-ligated to the *Eco*RI restriction site. This technique allows obtaining multiple fragments representing different copy locations of a particular LTR-RT in the genome

model and bootstrapping was performed after 1000 replicates under the appropriate amino acid substitution model (Figs. S1, S2). Primers were designed for retrotransposon families with evidence of recent insertion. Based on the mechanism of transposition of LTR-RTs, the two LTR sequences will be identical at the moment of insertion, hence, recently inserted elements will not present any differences between their LTR sequences (Finnegan 2012). For each identified LTR-RT, lower sequence divergence was determined as more than 99% similarity among its LTR sequence (Table S1) (Kibbe 2007; Untergasser et al. 2012). Ten LTR-RT were randomly selected to study transposon activation by S-SAP and qPCR techniques (Table S3).

Sequence-specific amplification polymorphisms (S-SAP)

Using the S-SAP technique, the effect of hybridization and polyploidization on the activation of LTR transposon was analysed in ten LTR-RT (Copia 1, Copia 12, Copia 14, Copia 21, Copia 17, Copia 84, Gypsy 6, Gypsy 16 and Gypsy 22). S-SAP is a modification of the AFLP technique adapted to detect polymorphisms associated with the mobility of retrotransposons and is very efficient to test mobilization of high copy number elements (Syed and Flavell 2006). Briefly, the DNA is digested with restriction enzymes that cut preferably outside the LTR element and the fragments generated in the restricted DNA are ligated to adapters and amplified by PCR with specific primers to the adaptor and the LTR sequence. Different unique fragments are generated based on the insertion site of the element and the adjacent restriction cut site (Fig. 1b).

DNA digestion was carried out in triplicate with 300 ng DNA, 2U of *Eco*RI (NEB, Massachusetts, USA), 2U of *Mse*I, 1.25 µL of CutSmart buffer (NEB, Massachusetts, USA) with 100 ng μL^{-1} BSA in a final volume of 12.5 µL and was incubated for 3 h at 37 °C. The quality of the digestion was checked by electrophoresis in a 0.8% agarose gel stained with ethidium bromide.

The ligation reaction was performed using 6.25 µL of the digest products, 1.25 µL 20 mM of *Eco*RI adaptors, 1.25 µL 20 mM of *Mse*I adaptors, 1.25 µL T4 DNA ligase buffer (Promega, Wisconsin, USA), 1U of T4 DNA ligase (Promega, Wisconsin, USA) in a final volume of 12.5 µL for 3 h at 20 °C.

Pre-amplification was performed using [*Eco*RI + 1] and [*Mse*I + 1] primers and each PCR contained 1 µL of the ligation products, 50 ng μL^{-1} of primers, 2 nM of dNTPs, 1X Taq DNA polymerase buffer, 1U of Taq DNA polymerase (Invitrogen, ThermoFisher Inc, Massachusetts, USA) in a final volume of 25 µL. The PCR protocol was as follows: 20 cycles at 94 °C for 30 s, 56 °C for 1 min, and 72 °C for 1 min. The pre-amplification products were diluted 1:3 with sterile ultra-pure water and were monitored using 8 µL of the reaction for electrophoresis on 1% agarose gel, stained with ethidium bromide, and visualized under UV light.

Selective amplification was carried out with the primer *Eco*RI + 1 and primers specific to each retrotransposon family (Table S2). For each reaction, 1 µL of the diluted pre-amplification, 50 ng μL^{-1} of primers, 2 nM of dNTPs, 1X Taq DNA polymerase buffer, 1U of Taq DNA polymerase (Invitrogen, ThermoFisher Inc, Massachusetts, USA) in a final volume of 20 µL. The amplification program used was: 14 cycles at 95 °C for 30 s, 55 °C for 30 s (decreasing 0.7 °C per cycle) and 72 °C for 1.5 min, followed by 30 cycles at 95 °C for 30 s, 52 °C for 30 s and 72 °C for 1.5 min.

The fragments obtained from the amplification were separated in polyacrylamide denaturing gels (6%). Three technical replicates were analysed to validate the procedure, and only reproducible patterns on all replicates were used for analysis. Fragments were scored as present (1) or absent (0), and then recorded and converted into a binary matrix.

For each model, the differences in fragment presence were evaluated. We considered any novel fragment as a novel transposition event, shared fragment as additivity, and loss fragments as segregation. For the hybridization model, we directly compared the hybrid (2xPIH) with ktz and tbr; for the allopolyploid model each allotetraploid lines (4xAL1, 4xAL2, 4xAL3, and 4xAL4) was compared with 2xPIH, and for the autotetraploid model each ktz-autotetraploid line (4xAuL1, 4xAuL2, and 4xAuL3) was compared with ktz.

Fragment recovery and sequencing

Novel fragments that were present in the interspecific hybrid but absent in the parental genotypes could be the result of LTR-RT activation and mobilization. To verify this possibility, these fragments of interest were recovered and re-amplified using the same primers and PCR cycle from the specific amplification. The products were separated by electrophoresis in 2% agarose gel, stained with ethidium bromide, visualized under UV light, and purified with QIAEXII gel extraction kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The purified PCR products were directly sequenced using Big Dye Terminator v3.1 kit (Applied Biosystems, ThermoFisher Inc, Massachusetts, USA) in an automated Genetic Analyzer 3130xl (Applied Biosystems, ThermoFisher Inc, Massachusetts, USA).

Genomic copy number quantification of LTR-RT

We performed real-time quantitative PCR (qPCR) on DNA samples using primers to amplify a partial sequence of the reverse transcriptase gene (*RT*-gene) (Table S2) and estimate the copy number of LTR-RTs. The results were normalized to the single-copy number gene *ATG-1*, a gene used as a reference in previous copy number analysis (Segura et al. 2017).

In order to obtain the *RT*-gene plasmid for the standard curve, we performed the amplification of each *RT*-gene with Taq DNA polymerase using specific primers (Table S2); the PCR products were separated by electrophoresis on 2% agarose gel, stained with ethidium bromide, and visualized under UV light. The specific amplification products were recovered and purified with the QIAEXII gel extraction kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions and quantified by spectrophotometry. Amplified fragments were cloned into pCR2.1 TOPO vector (Thermo Fisher Inc, Massachusetts, USA). The insertion of

the amplified product was checked by digestion with *Eco*RI and PCR, using M13 primers. Standard curves were carried out with serial dilutions of the plasmid and the number of copies of each amplicon was calculated based on the plasmid amount (ng) and the length of the DNA sequence (http://scienceprimer.com/copy-number-calculator-for-realtime-pcr). Since primers were designed for the RT-gene absolute the qPCR could also amplify extrachromosomal DNA of active LRT-TR.

Besides the standard curve, 25 ng of the DNA samples were used for qPCR employing the Mezcla Real Master-Mix (Cat. no. B124-100, Biodynamics SRL, Buenos Aires, Argentina) with the corresponding primers. The amplified products were quantified by fluorescence using StepOnePlus (Applied Biosystems, ThermoFisher Inc, Massachusetts, USA). Cycling conditions were optimized for each primer and a melting curve stage was added (Table S1).

RT-gene expression

Transcription activity of Copia 12, Copia 14 and Copia 27 was analyzed in two 2xPIH biological replicates by absolute qPCR. Briefly, RNA was extracted from leaves by duplicates using TRIzol method according to the user manual (Invitrogen, ThermoFisher Inc, Massachusetts, USA). Concentration and purity were determined using the DS-11 Spectrophotometer (DeNovix Inc., Wilmington, USA). 100 ng of RNA was used for reverse transcription using 200 U M-MLV Reverse Transcriptase (Invitrogen, ThermoFisher Inc, Massachusetts, USA) according to the user manual and 2 ng Random Primers (Cat. no. B070-40, Biodynamics SRL, Buenos Aires, Argentina) for cDNA amplification. To verify the quality of the cDNA, a PCR was performed using the Elongation Factor 1-alfa (EF1-alfa) primers (Forward primer: 5'ATT GGAAACGGATATGCTCCA3', Reverse primer: 5'TCC TTACCTGAACGCCTGTCA3'). For each reaction, 1 µL of the 50 µg cDNA, 50 ng μL^{-1} of primers, 10 nM of dNTPs, 1X Taq DNA polymerase buffer, 1U of Taq DNA polymerase (INBIOHighway, Buenos Aires, Argentina) in a final volume of 15 µL was used. The amplification program used was: 95 °C for 5 min, 35 cycles at 95 °C for 30 s, 54 °C for 30 s and 72 °C for 1 min, followed by 72 °C for 7 min. The 100 pb products were separated by electrophoresis in 2% agarose gel, stained with ethidium bromide and visualized under UV light (Fig. S5). The absolute qPCR of the *RT*-gene of Copia 12, Copia 14 and Copia 27 was performed using 250 ng of cDNA along with their respective standard curve as mentioned in the previous section.

Statistical analysis

For S-SAP analysis, we present the results in the form of an UpSet plot using the package 'UpSetR' in R version 4.0.3 (Lex et al. 2014; Conway et al. 2017; R Core Team 2020). To statistically compare the activation of LTR-RTs, three models were considered (2xPIH, allotetraploid, and ktz-autotetraploid) and analysed with a Poisson log-linear model using the package stats in R (R Core Team 2020). The dice coefficient was calculated using the binary matrix generated from all S-SAP data and the genetic distance was represented in a dendrogram (InfoStat version 2018 software; Grupo InfoStat, Argentina).

For genomic normalized copy number quantification of LTR-RTs, differences were calculated using a log-normal heteroscedastic model, followed by planned orthogonal contrasts using nlme and multcomp packages in R (Hothorn et al. 2008; Pinheiro et al. 2021). In order to compare the data from S-SAP and qPCR analyses we performed Mantel test, Procrustes analysis, and comparative clustering was visualized using a tanglegram, as implemented in ade4, vegan and dendextend packages (Galili 2015; Oksanen et al. 2020; Thioulouse et al. 2018).

Results

Identification of putative active LTR-RTs

A total of 47 unique sequences annotated as LTR-Gypsy and 97 as LTR-Copia were identified. From these, three LTR-Gypsy and 35 LTR-Copia elements presented 100% identity between their LTRs, indicating recent insertion in the potato genome and being potentially active (Table S1). The majority of the putative active LTR-Copia belonged to the retrofit family (Fig. S2); ten of which were selected to test their activation in the potato hybrid and tetraploid lines (Table S1).

LTR-RT activity in potato genome

In our models (Fig. 1), we evaluated the specific TE activation comparing the amplification of fragments through S-SAP technique among (i) the interspecific hybrid 2xPIH and its parental species, tbr and ktz, (ii) four allotetraploid (4xAL1, 4xAL2, 4xAL3 and 4xAL4) and the 2xPIH parental line and (iii) three ktz-autotetraploid (4xAuL1, 4xAuL2 and 4xAuL3) lines of ktz.

We analyzed ten LTR-RT, seven Copia and three Gypsy (Table 1). A total of 705 fragments were analysed in acrylamide gels. The hybrid 2xPIH showed 29 novel fragments (present in the hybrid but absent in the parental species, tbr and ktz) that were also present in the allotetraploid lines (see Fig. 2). Only one novel fragment was detected in two independent autotetraploid lines (AuL1 and AuL2). The UpSet plot shows the number of fragments shared between parental, hybrid, and derived polyploid genomes for each LTR-RT, (Fig. 2). The number of amplified fragments for

Table 1 Sequence-specific amplification polymorphisms (S-SAP) amplified fragments in the interspecific hybrid 2xPIH and its parental species, *Solanum kurtzianum* (ktz) and *Solanum tuberosum* (tbr)

LTR-RT	Number of fragments					Fragment dynamic in 2xPIH		
	Total	ktz	tbr	2xPIH	Shared (ktz-tbr)	Lost from ktz (%)	Lost from tbr (%)	Novel fragments (%)
Copia 1	56	31	49	46	26	1(3.2)	8 (16.3)	2 (4.3)
Copia 12	89	61	70	80	47	0 (0)	9 (12.9)	5 (6.3)
Copia 14	55	42	37	46	29	5 (11.9)	4 (10.8)	5 (10.9)
Copia 21	116	90	90	103	65	5 (5.5)	8 (8.9)	1 (1.0)
Copia 27	82	55	50	71	31	6 (10.9)	5 (10.0)	8 (11.3)
Copia 62	48	41	29	34	23	9 (22.0)	5 (17.2)	1 (2.9)
Copia 84	55	27	49	43	23	2 (7.4)	9 (18.4)	1 (2.3)
Gypsy 6	61	44	55	52	38	2 (4.5)	7 (12.7)	0 (0)
Gypsy 16	48	38	29	42	21	4 (10.5)	2 (6.9)	2 (4.8)
Gypsy 22	80	58	58	68	40	4 (6.9)	8 (13.8)	4 (5.9)

Ten LTR-RT families from the Copia and Gypsy superfamilies were analysed

each evaluated LTR-RT varied from 48 to 118 (Table 1). The hybrid shared on average 87% of fragments with its progenitors, while between ktz and tbr parental genotypes shared on average 53% of fragments (Table 1). The novel fragments, i.e. present in the hybrid but absent in the parental genotypes, represented between 0 and 11.3% of total S-SAP loci (Fig. 2, Table 1) and were considered as novel insertion events as a result of LTR-RT activation. The LTR-RTs with major activity were Copia-27, Copia-12, Copia-14, and Gypsy-22 with 8, 5, 5, and 4 novel fragments, respectively. The proportion of fragments presented in the parents but absent in the hybrid varied from 0 to 22% for ktz and from 6.9 to 13.8 for tbr (Table 1). Based on the mobilization analysis by S-SAP of the selected LTR-RT elements, we found that 9 out of 10 elements were activated in 2xPIH and present in the allotetraploids, while only one novel element (Copia-84) was detected in two ktz-autotetraploid lines (4xAuL1 and 4xAuL2), and none were activated in the allotetraploid lines (Fig. 2). The statistical analysis of the number of novel fragments in each genotype revealed that there were no differences between TEs and that the activation of TEs in 2xPIH was statistically significant ($P < 0.001$). The mean number of novel fragments was 2.8. In the polyploid model, there was no significant activation in 4xAL ($P = 0.996$), whereas 4xAuL showed a mean number of novel fragments significantly greater than zero ($P < 0.05$). Upon closer inspection of the statistical model, this was the effect of the unique novel fragment from Copia-84 present in 4xAuL1 and 4xAuL2 as previously mentioned, which is an outlier in the model, and the mean number of novel fragments was just 0.036.

Cluster analysis of S-SAP fragments from allo-, ktz-autotetraploids and parental lines (Fig. 3) showed that polyploids clustered with their diploid parental lines. Allo- and ktz-autotetraploid lines shared more than 99.9% of S-SAP fragments with 2xPIH and ktz, respectively. Therefore, no evident LTR-RT activation was detected after whole-genome duplication.

To investigate the genome context where the novel LTR-RTs copies were inserted in 2xPIH, we re-amplified and sequenced the novel fragments present in the interspecific hybrid of the Copia-27, Copia-12, Copia-14, and Gypsy-22 LTR-RTs. A total of 7 fragments were confirmed and characterized (Table 2). The insertion site was variable, ranging from genes to other LTR sequences and unclassified regions.

To evaluate if the LTR-RT are transcriptionally active in the 2xPIH, we analysed the *RT*-gene expression in three LTR-RT with major S-SAP activity (Copia 12, Copia 14 and Copia 27) by absolute qPCR. We did not detect expressions of Copia 14 and Copia 27, only Copia 12 presented low expression and approximately 20 to 25 copies were estimated.

LTR-RT copy number quantification

Possible activation of LTR-RT after polyploidization was also assayed by quantification of LTR-RT copy number. We designed reverse transcriptase (*RT*-gene) primers (Table S1) for the four families with major S-SAP activity (Copia-27, Copia-12, Copia-14, Copia-27 and Gypsy-22) and performed an absolute quantification by qPCR in the ktz-auto and allotetraploid lines and their diploid parental lines ktz and 2xPIH, respectively. We normalized the results with the single-copy gene *ATG-1* to avoid the variation originated by the genome duplication per se. As shown in Fig. 4, no statistical differences were detected between ktz-autotetraploid lines and their progenitor in the four LTR-RTs analysed. The same results were obtained comparing the allotetraploid lines with their progenitor (2xPIH), except for Copia-12 ($P < 0.05$).

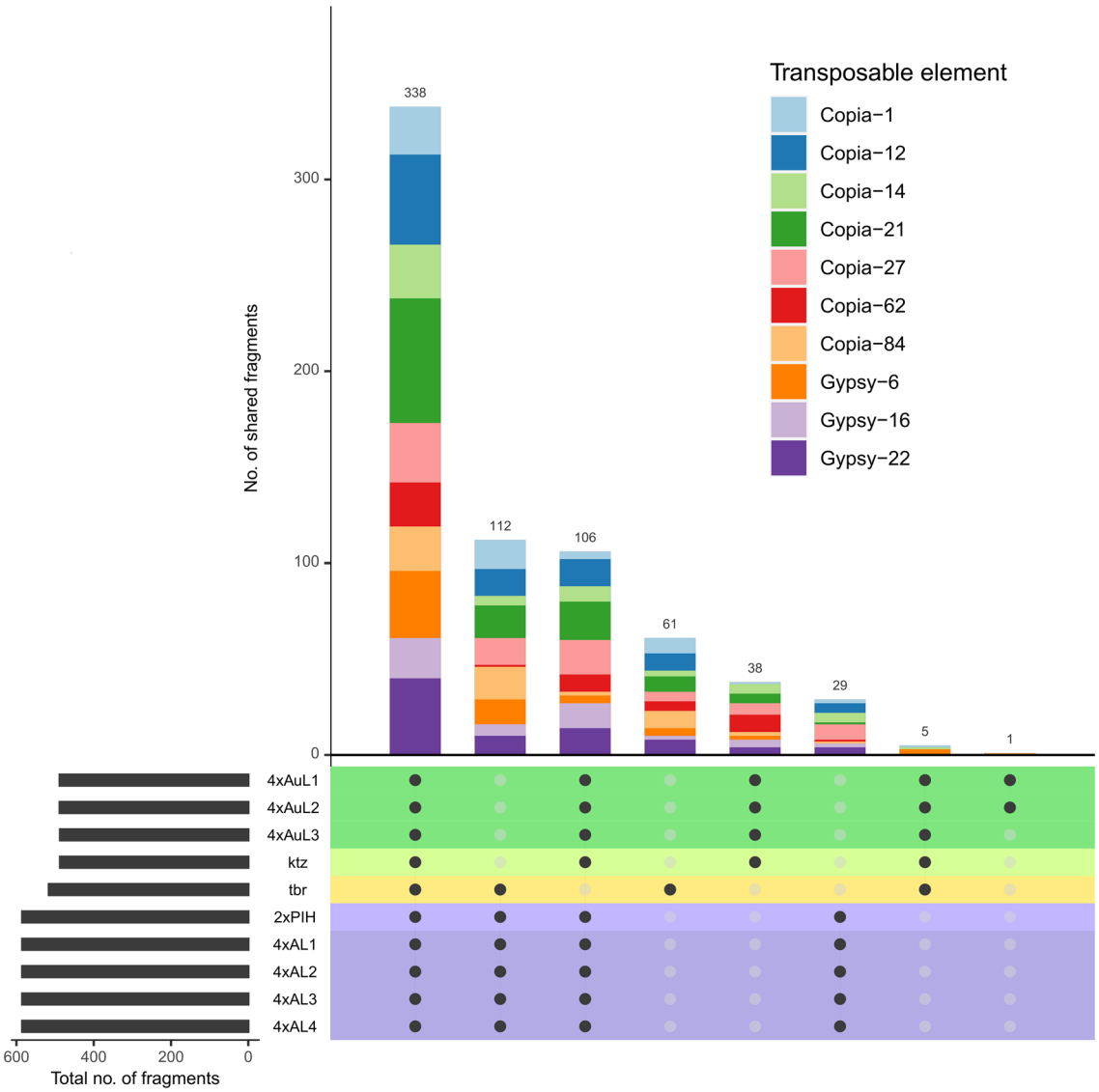

Fig. 2 S-SAP analysis of diploid parental species *Solanum kurtzianum* (ktz) and *Solanum tuberosum* (tbr) and the interspecific hybrid (2xPIH). The UpSet plot shows the total number of S-SAP fragments detected in *S. tuberosum* (tbr, yellow), *S. kurtzianum* (ktz, light green), ktz-autotetraploid (4xAuL, green), interspecific hybrid (2xPIH, light purple), and allotetraploid lines (4xAL, purple) in the horizontal barplot. The vertical barplot represents the individual fragments of 10 LTR-RT elements shared between samples. Unique fragments present in 2xPIH and 4xAL lines but absent in the parental species tbr and ktz are interpreted as hybridization induced active LTR-RTs while those present in either one or both parents but absent in the hybrid represent fragment lost

These results are in concordance to those obtained by the S-SAP technique, *i.e.* no TE activation was evidenced after polyploidization in the potato evaluated genomes. Mantel test showed a significant ($P < 0.001$) and high correlation between S-SAP and qPCR matrices (0.87) based on 9999 replicates (Fig. S3). Procrustes analysis also showed significant ($P < 0.001$) and high correlation on a symmetric rotation (0.46) on 9999 permutations (Fig. S4). The comparison of the dendrograms obtained using S-SAP and qPCR data in a tanglegram show high concordance between datasets, as in both, the lines of each model clustered together in highly homogeneous clusters (Fig. S5).

Discussion

Hybridization and whole-genome duplication events are common in plant evolution and have been recognized as important speciation mechanisms. TE amplification and polyploidization are considered the most important mechanisms responsible for increasing plant genome size, which is

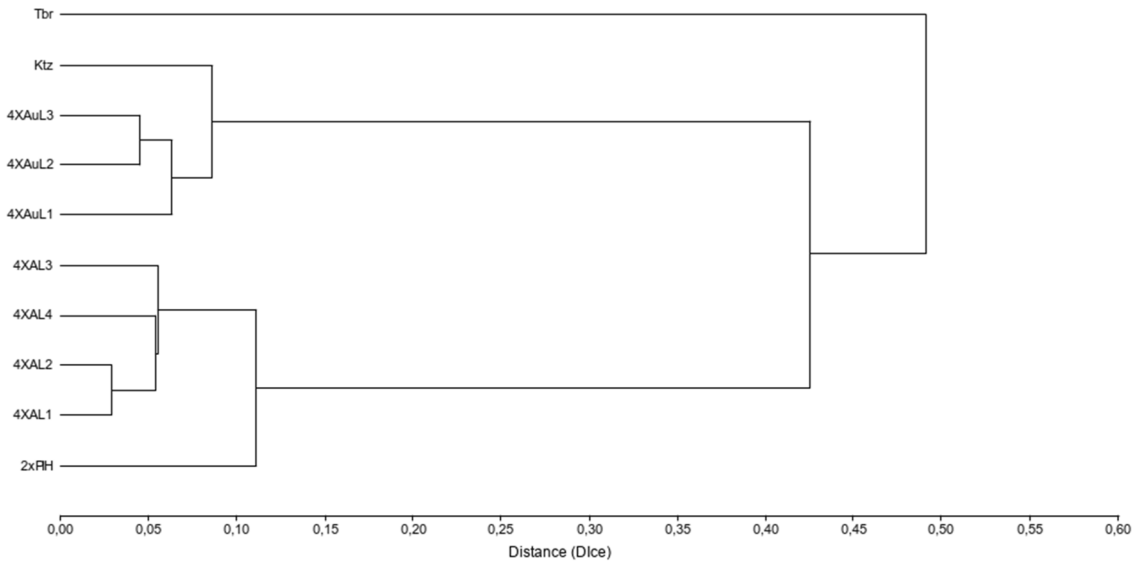

Fig. 3 Distance based on Dice coefficient. Dendrogram obtained by cluster analysis based on presence/absence of the S-SAP profiles of 10 LTR-RT (Copia-1, Copia-12, Copia-14, Copia-21, Copia-27, Copia-62, Copia-84, Gypsy-6, Gypsy-16, and Gypsy-22) in the parental species (*Solanum tuberosum* and *Solanum kurtzianum*), the diploid interspecific hybrid (2xPIH), four derived allotetraploid lines (4xAL1, 2, 3 and 4) and three derived ktz-autotetraploid lines (4xAuL1, 2 and 3)

Table 2 LTR-RT insert location of novel fragments in one interspecific hybrid between *Solanum tuberosum* and *Solanum kurtzianum* detected by S-SAP

LTR-RT	Fragment	Insertion site	Location in the potato reference genome (GCF_000226075.1)		
			Chromosome number	Start	End
Copia 12	C12-3-H3	PPR-Domain	5	13,415,759	13,415,617
Copia 14	C14-1-H3	Rtgene-Gypsy25	1	55,334,149	55,334,310
Copia 14	C14-3-H3	LTR-Gypsy41	1	44,593,802	44,593,915
Copia 27	C27-2-H3	Unknown function	3	12,416,002	12,416,151
Copia 27	C27-3-H3	Beta-glucosidase 11-like	9	56,602,802	52,602,614
Copia 27	C27-6-H3	Unknown function	11	3,885,030	3,885,090
Gypsy 22	G22-1-H3	Unknown function	11	18,453,168	18,452,900

an important event in plant genome evolution (Casacuberta and González 2013; Vicient and Casacuberta 2017; Wendel et al. 2016). It was proposed that TE bursts could be a consequence or concomitant to genome duplication or hybridization. Investigations in various polyploid systems showed that polyploidization, principally allopolyploidization, was associated with changes in genome structure, DNA methylation, TE activation, and gene expression (Marfil et al. 2018; Yaakov and Kashkush 2010). Activation of specific LTR-RT families has been reported in *Solanum* species. For example, the LTR-RT Tnt1 was initially characterized in *Nicotiana tabacum* and then detected by probe hybridization in other *Solanaceae* genomes (Grandbastien et al. 1989, 2005). Manetti et al. (2009) revealed the existence of a lineage-specific dynamic flux regarding the history of the amplification of Tnt1-like elements in the genome of *Solanum* species (Manetti et al. 2009). In line with these studies, the assessment of genetic diversity among accessions of the cultivated potato showed the widespread presence and distinct DNA profiles for Copia-like and Gypsy-like LTR-RT in different genotypes, indicating that these elements are active in the genome and may have even contributed to the potato genome organization (Sharma and Nandineni 2014). Activation of Tnt1 and Tto1 Copia retrotransposons were detected in interspecific hybrids between the wild potato species *S. kurtzianum* and *S. microdontum* (Paz et al. 2015). In other species like pepper (*Capsicum annuum*), TE amplification has happened without whole-genome duplication, increasing the genome size. In *C. annuum*, constitutive heterochromatin was actively expanded 20.0–7.5 million years ago through a massive accumulation of single-type Ty3/Gypsy-like elements that belong to the Del subgroup (Park Minkyu et al.

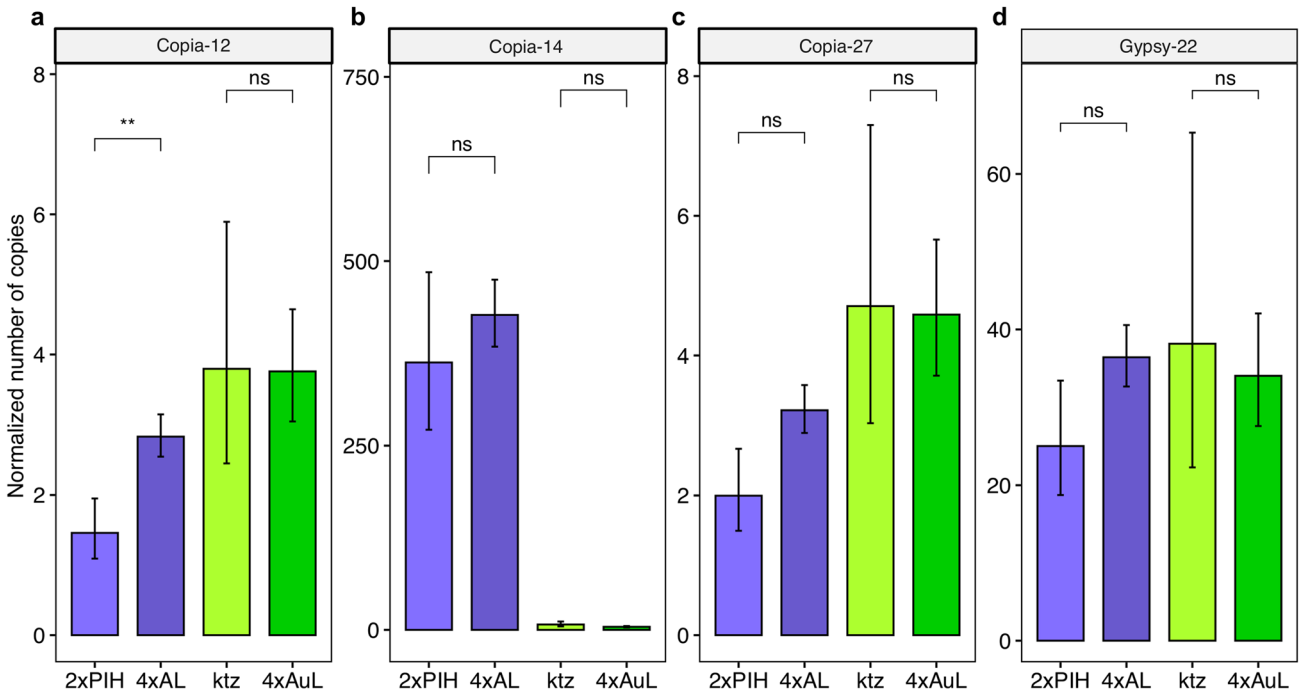

Fig. 4 Mean normalized copy number and 95% Confidence Interval (CI) of **a** Copia-12, **b** Copia-14, **c** Copia-27 and **d** Gypsy-22 LTR-RTs in allotetraploid lines (4xAL), diploid interspecific hybrid genotype (2xPIH), ktz-autotetraploid (4xAuL) and diploid ktz parental genotype (ktz). To avoid over-estimation associated with ploidy, absolute LTR-RT copy number was normalized using the single-copy gene ATG-1. No statistically significant differences were detected between polyploid lines and their diploid parental lines except between 2xPIH and 4xAL in Copia-12

2011). Recently, Esposito et al. (2019) and Zavallo et al. (2020) estimated that in *S. tuberosum* and *S. commersonii* genomes the LTR-RT Copia were younger than Gypsy elements with insertion times of approximately 2 and 4 Mya, respectively. Our identification and phylogenetic analyses support the idea that proliferation occurs in specific families of LTR-RTs, as we observed a major proportion of activation in specific families, e.g. retrofit family, belonging to the Copia superfamily (Fig. S1). Most of the youngest LTR-Copia sequences belong to the retrofit family, which suggests recent amplification of this clade in the potato genome. The particular amplification of LTRs and their association with a specific plant section or species points out that retroelement expansion within a genome is unique and depends on the evolutionary processes within each plant species (Paz et al. 2017).

Our S-SAP study of ten LTR-RT in the interspecific hybrid and its parental species highlight the specific response of transposons activation in potato; Copia-27, Copia-12, Copia-14, and Gypsy-22 showed more than four novel fragments in the hybrid. In contrast, only one change was observed after polyploidization in the ktz-autotetraploid line. Similar to our results, several reports showed that there was a limited transpositional activity of TEs in synthetic polyploids of Arabidopsis (Madlung et al. 2005), *Spartina* (Parisod et al. 2009) and wheat (Kashkush et al. 2003). In our model, after hybridization, there were several generations of clonal propagation and it is possible that the TE bursts produced by hybridization subsided in advanced generations of clonal propagation (Madlung et al. 2005). The genome remodeling during clonal propagation could excise some immediate TE activation after hybridization or polyploidization. At the same time, if the LTR-RT is still active after the hybridization, as seems to be the case for Copia 12, some of the changes that we are reporting in the 5th generation could be the result of the cumulative changes by the generations.

Our results are in concordance with those reported by Mhiri et al. (2018) in *Nicotiana* hybrids and allotetraploids. In the same way, Parisod et al. (2010) suggested that TE proliferation in the short or the long term after allopolyploidization may be restricted to a few TEs, and also in specific polyploid systems. In *Brassica*, Sarilar et al. (2013) studied and compared S-SAP profiles between resynthesized *Brassica napus* allo-tetraploids and their diploid progenitors. No massive structural changes associated with TEs were detected. Their study supports the idea that TE responses to allopolyploidy are highly specific (Sarilar et al. 2013).

An exhaustive review by Vicient and Casacuberta (2017) indicated that polyploidization could induce bursts

of transposition as a result of the relaxation in the epigenetic control of TEs. The authors suggest that the TE bursts could lead to major genome changes which include: recombination, loss of regions and chromosome rearrangements (Vicient and Casacuberta 2017). However, the complete scenario of allopolyploid formation includes parental species, hybrids, and polyploids; so, most of the activation observed in several allopolyploid systems could be a result of hybridization. The hybridization step (i.e. the merge of differentiated complete genomes in one single cell), constitutes a major 'genome shock', as first hypothesized by McClintock (1984). This 'genome shock', usually accompanied by structural and functional modifications, has been associated with the activation of TE. As mentioned by several authors, the genome imbalance of TE could influence the interaction of subgenomes and their epigenetic control (Lim et al. 2004; Mhiri et al. 2018; Michalak 2009; Parisod et al. 2012), enabling some specific LTR-RTs to escape the genetic repression. In our model, the parental species used are closely related, as both belong to the *Tuberosa* series and have highly homologous genomes (Matsubayashi 1991). Therefore, studies including species from different series of the section *Petota* may show higher TE activation. In previous work, methylation-sensitive amplification polymorphism (MSAP) changes were observed, suggesting that alteration of methylation status is a common phenomenon in these interspecific hybrids (Marfil et al. 2006) and could be influencing the activation of specific LTR-RTs.

Being one of the most important crops worldwide and having rich related germplasm with different ploidy levels, the cultivated potato constitutes an excellent model to study TE activation induced by polyploidization and hybridization. This is the first report that compares TE mobilization in newly synthesized allo- and ktz-autotetraploid potatoes. We found moderate activation of specific TE families, like Copia and Gypsy, induced by hybridization. As shown in previous studies in other species, based on our results, polyploidization per se would not be relevant to LTR-RT activation in potato.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10265-021-01354-9.

Acknowledgements This work was supported by the following institutions from Argentina, Agencia Nacional de Promoción Científica y Técnica (ANPCyT) PICT 1243 and Universidad Nacional de Cuyo, Mendoza, Argentina, project 06/A597. G.M, M.A and I.V.N. are Consejo Nacional de Investigaciones Científica y Técnicas (CONICET) fellows.

Declarations

Conflict of interest The authors declare no competing interests.

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