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The influence of mismatch repair deficiency in somatic hybrids between *Solanum tuberosum* + *Solanum chacoense* and genome editing of *MSH2* gene in potato

Ph.D. thesis summary

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"We totally missed the possible role of ... [DNA] repair although ... I later came to realize that DNA is so precious that probably many distinct repair mechanisms would exist."

(Crick, 1974)

Chapter 1. An Overview of the literature

1.1. Potato and its wild relatives as a source of resistance genes

The cultivated potato (*Solanum tuberosum* L.) is the third most important staple food crop in the world after rice and wheat in terms of human consumption (https://cipotato.org/).

The cultivated potato has narrow genetic germplasm resulting initially from a limited population, which was brought to Europe and has suffered a high inbreeding depression due to the intensive breeding (Ghislain and Douches, 2020).

The potato tubers present an important dietary source of starch, protein, antioxidants, and vitamins; simultaneously, they serve the plant as a storage organ and as a vegetative propagation system (Burlingame et al., 2009). Cultivated potato and its wild relatives belong to the genus *Solanum*, the largest genus with 1,500–2,000 species (Machida-Hirano, 2015). The wild tuber-bearing potatoes are called *Solanaceae* sect. *Petota* Dumort. (*Solanum* L. species) and the outgroup relatives which did not produce tubers, *Solanum* sect. *Etuberosum* (Bukasov and Kameraz) Child, are relatives of the cultivated potato (Hawkes, 1990; Spooner et al., 1993).

In addition to these cultivated potato species, there are 199 wild potato species (*Solanaceae* sect. *Petota* Dumort) found sporadic in 16 countries from the southwestern United States to central Argentina and Chile (Hijmans and Spooner, 2001).

Wild potatoes are promising sources of desirable agricultural traits for the breeding of cultivated potato. (Jansky et al., 2009; Pelletier et al., 2011) They possess good tolerance against abiotic stress like heat, among other resistance traits (e.g. *S.berthaultii*, *S. chacoense*, and *S. stoloniferum*) (Reynolds and Ewing, 1989; Guedes et al., 2019). In *S. berthaultii* Hawkes and in *S. bulbocastanum* Dun., resistance genes against *Phytophthora infestans* were found (Ewing et al., 2000; Naess et al., 2000, 2001; van der Vossen et al., 2003). *S. brevidens* has a broad virus resistance (Valkonen et al., 1994; Rokka et al., 1998).

S. chacoense present in his germplasm resistance to potato virus A and Y (PVA and PVY), late blight, Colorado potato beetle (CPB), tuber moth, potato leafroll virus (PRLV) (Brown and Thomas, 1994; Hawkes, 1994) and resistance to cold-induced sweetening (Leisner et al., 2018).

The wild relatives of potato present a priority for conservation because they possess high genetic diversity useful for developing more productive, nutritious, and resistant crop varieties (Castañeda-Álvarez et al., 2016). The ability to cross the wild species with the cultivated species

is essential and is based on the endosperm balance number (EBN) and the ploidy (Jansky et al., 2009). Moreover, to cross a wild potato with the cultivated one is very challenging because they possess unilateral incompatibility and self-incompatibility. Besides this, they possess different EBN and ploidy, reviewed in Spooner et al., 2014. Furthermore, somatic hybridization is used to overcome the incompatibility between wild and cultivated potatoes, often being the only way to introgress important resistance traits from the wild *Solanum* species into the cultivated potato germplasm (Xu et al., 1991; Chen, 2004; Chen et al., 2008; Thieme and Rakosy-Tican, 2017).

Accelerate climate change has a significant impact on potato production, and much more insecticides and pesticides are required to control diseases and pests. Large scale application of chemical pesticides can lead to serious health and environmental problems (Alyokhin, 2008; Maharijaya and Vosman, 2015). Mild winters offer very favorable conditions for survival for CPB and as well for other pests.

Chapter 2. Goals and objectives of the thesis

In order to introgress valuable traits from the wild relatives, an MMR deficiency was introduced in *S. chacoense*. The MMR-deficient *S. chacoense* was used in somatic fusions with the tetraploid cultivated potato (Rakosy-Tican et al., 2004; Rakosy-Tican et al., 2019).

This work aimed to characterize the potato somatic hybrids between *S. tuberosum* and *S. chacoense* with a deficiency in the MMR system. Furthermore, to exhibit if MMR deficiency favour homeologous recombination in meiosis of the SHs in order to introgress resistance traits from *S. chacoense.*

The next step was the generation of MMR-deficient potato plants using the new genome editing technique (CRISPR/Cas9) with *Agrobacterium tumefaciens* and the DNA-free delivery method with PEG in potato protoplasts, as a proof of concept.

The objectives of this thesis were:

1) The analysis of somatic hybrids for mutant phenotypes, MSI in transgenic and control (parent) plants, resistance to kanamycin as a marker of the transgenic construct integration and RTq-PCR for *MSH2* and *SPO11* genes in selected somatic hybrids and their parents.
2) The characterization using GISH of homeologous recombination in meiosis, and pollen viability analysis in selected SHs.
3) The determination of resistance to Colorado potato beetle of selected SHs with or without MMR deficiency.
4) The generation of MMR-deficient potato plants using the new genome editing technique (CRISPR/Cas9) mediated by *Agrobacterium tumefaciens* transformation or the DNA-free delivery method in potato protoplasts using PEG. This work aimed to establish the method for genome editing in potatoes in order to use the know-how to edit further resistance genes and later to knock-out the transgene from the SHs using the DNA-free genome editing method. It is crucial to have transgene-free plants because of the regulatory perspective.

Chapter 3. Plant material used in this thesis

To introgress the resistance genes from the wild relative of potato, *S. chacoense*, genetic transformation with *A. tumefaciens*, strain LBA4404 was done (Rakosy-Tican et al., 2004; Rakosy-Tican et al., 2019). The constructs used for genetic transformation, the AS (antisense) construct, and the DN (dominant-negative) construct are described in the following paper: (Rakosy-Tican et al., 2004, 2019).

The antisense construct (AS) contains the 3' 1 kb fragment from *A. thaliana MSH2* cDNA in an antisense orientation (*Figure 1- A*), and inhibition of the *MSH2* gene occurs through antisense strategy. The dominant-negative construct is an *AtMSH2* coding sequence (*Figure 1- B*), which contains a point mutation at position 697 where a highly conserved Asp (aspartate) codon is changed in Gly (glycine) codon (Ispas, 2004) and inhibition of *MSH2* gene occurs through competitive inhibition. The same mutation in the *MSH2* gene, introduced in yeast at a homologous position confers a strong dominant-negative phenotype (Nicolaides et al., 1998; Studamire et al., 1999). The transgenic status of the clones is described in: (Rakosy-Tican et al., 2004).

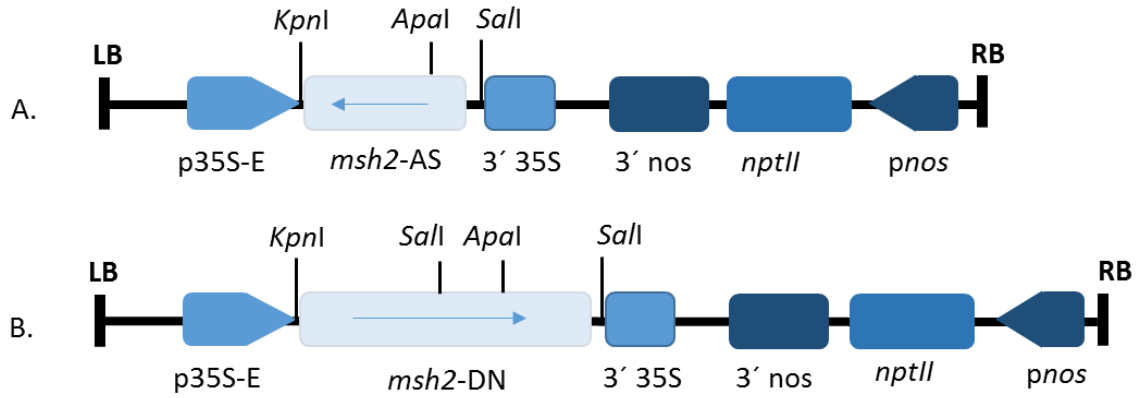

Figure 1. Schematic representation of the plasmids transformed into *S. chacoense*, **(A)**T-DNA of the FRG-*MSH*2-AS plasmid, **(B)** T-DNA of the FRG-*MSH2*-DN plasmid (Rakosy-Tican et al., 2004, 2019).

Two potato cultivars, cv. Delikat (Nordring-Kartoffelzucht – und Vermerungs – GmbH Gross Lüsewitz, Germany) and cv. Desiree (ZPC, Leeuwarden, Netherlands) were used to generate somatic hybrids between *S. tuberosum* and the *MSH2* transgenic *S. chacoense* (Rakosy-Tican et al., 2004, 2019). The hybridity of these SHs was analysed using SSR markers (data not shown here). In this thesis, the SHs without the transgenic construct is coded using numbers (i.e., SH 1837/1) or the control with C followed by a number (i.e., DkC 5; DeC 7). The potato cultivars' names are shortened as Dk = cv. Delikat and De = cv. Desiree. The *S. chacoense* is shortened *S.*

chc or *chc*. The somatic hybrids, which were transformed with the dominant-negative mutant *MSH2* gene, are labelled as DkDN 5, DkDN 11, DeDN 5, and DeDN 11, with the numbers followed by the number of each clone. When the antisense (AS) construct was used to produce stable transformed SHs, then the clone number follows DkAS 10 (Rakosy-Tican et al., 2019).

The SHs with MMR deficiency was produced to increase homeologous recombination, therefore to increase the introgression of resistance genes toward CPB. MSH2 protein has an essential role in suppressing recombination between diverged sequences.

For the genome editing approach, tetraploid *S. tuberosum* cv. Delikat was used.

In bacteria (Rayssiguier et al., 1989; Zahrt and Maloy, 1997), yeast (Datta et al., 1996; Datta et al., 1997; Negritto et al., 1997; Chen and Jinks-Robertson, 1999; Nicholson et al., 2000), mammalian cells (Wind et al., 1995; Elliott and Jasin, 2001) and plants (Trouiller et al., 2006; Lafleuriel et al., 2007; Tam et al., 2011; van Marcke and Angenon, 2013), high recombination frequency was observed between homeologous sequences when the *MSH2* gene was suppressed or knocked-out.

Chapter 4. Results and discussions

4.1. New potato phenotypes co-induced by deficient *MSH2* and somatic hybridization

The majority of the work from **chapter 4.1** is already published in:

Rakosy-Tican, E.*, **Lörincz-Besenyei, E.***, Molnár, I.*, Thieme, R.*, Hartung, F., Sprink, T., Antonova, O., Famelaer, I., Angenon, G. and Aurori, A.* (2019). New phenotypes of potato co-induced by mismatch repair deficiency and somatic hybridization. Front Plant Sci 10, 3. doi: 10.3389/fpls.2019.00003. ***equal contribution**,

and in:

Molnár, I., **Besenyei, E.**, Thieme, R., Thieme, T., Aurori, A., Baricz, A., et al. (2017). Mismatch repair deficiency increases the transfer of antibiosis and antixenosis properties against Colorado potato beetle in somatic hybrids of *Solanum tuberosum* + *S. chacoense*. Pest management science 73, 1428–1437. doi: 10.1002/ps.4473.

4.1.1. MMR deficiency induces mutator phenotype in somatic hybrids

In *MSH2* defective *A. thaliana,* mutation accumulation was observed during seed-to-seed propagation, which goes hand in hand with different phenotypic mutations. In these plants, near-true albino leaves, early flowering, sterility, and dwarfism were described showing mutator phenotype (Hoffman et al., 2004).

To assess the mutator phenotype caused by the MMR deficiency and the variations caused due to somatic hybridization, the size of the plants, leaves, and internodes were analysed. MSI is a pattern of MMR deficiency, only the SHs with phenotypic abnormalities and MSI were considered to have a mutator phenotype caused by MMR deficiency, the other mutants are caused by somatic hybridization and its complex genetic interactions (Harms, 1983).

Phenotypic variations were observed in the SHs produced with the transgenic S. *chacoense* AS, DN, and potato cv. Delikat. Often, these SHs was showing large growth and dwarf phenotype (*Figure 3*). Moreover, abnormalities in leaf morphology, showing round shape, small leaves (*Figure 2***),** and lack of flower development as compared to the wild type parents were observed.

Dwarf SHs could be caused by mutations in plant hormone biosynthesis pathway or hormone receptors (Koorneef et al., 1985; Vega et al., 2006).

In one SH (DkDN 5.25) which contains the DN construct, plant shows disorganized grana with structural changes in the chloroplasts and thick inner cell wall around the stoma of the guard cells and these changes goes hand in hand with significant changes in leave structure having slightly greyish-green color and deformed leaves (*Figure 2***)**. Recently it was shown that the mitochondrial MSH1 protein associates also with the thylakoid membrane and a depletion cause variegation, curly and wrinkled leaf, abiotic stress tolerance, and deficient growth (Virdi et al., 2016). Nevertheless, the transcriptome of this somatic hybrid was analyzed in comparison with the wild type parent plants (cv. Delikat and *S. chacoense*), and the transcriptional profile of the Dk DN 5.25 is significantly different from cv. Delikat, and *S. chacoense*. Giant phenotype could be observed in the case of the hybrids (DkDN 5.3, 5.6, 5.11; DkDN 11.24 and 11.34) along with dwarf genotypes (DkDN 5.4, 5.17 and DkAS 10.20) which are significantly different from the parent plants.

Leaf size, measured at the midrib is significantly different from the parent plants in SHs with the MMR deficiency. Besides this, the internodes from the SHs with MMR deficiency shows different length. Dark purple and small tubers were observed as well in the mutator phenotypes. The analysed DN SHs show MSI in 82 % (9 genotypes from 11), and 45 % (5 genotypes from 11) shows mutator phenotype caused by MMR deficiency. However, the SHs generated with the AS mutation show MSI in 11 % (1 from 11 genotypes), and 11% shows the mutator phenotype.

The dominant-negative mutation is very effective in the case of these potato hybrids. This was observed in the case of the tomato, where the dominant-negative AtMSH2-DN protein construct showed an increase in homeologous recombination (Tam et al., 2011).

Some of the described somatic hybrids which have mutator phenotype do not develop flower, or the flowering occurred earlier as in parental plants. In *MSH2* defective tomato, floral abnormalities were observed in the first and second generation, which resulted to an abnormal stamen morphology and fruits without seeds or seeds with low viability (Sarma et al., 2018).

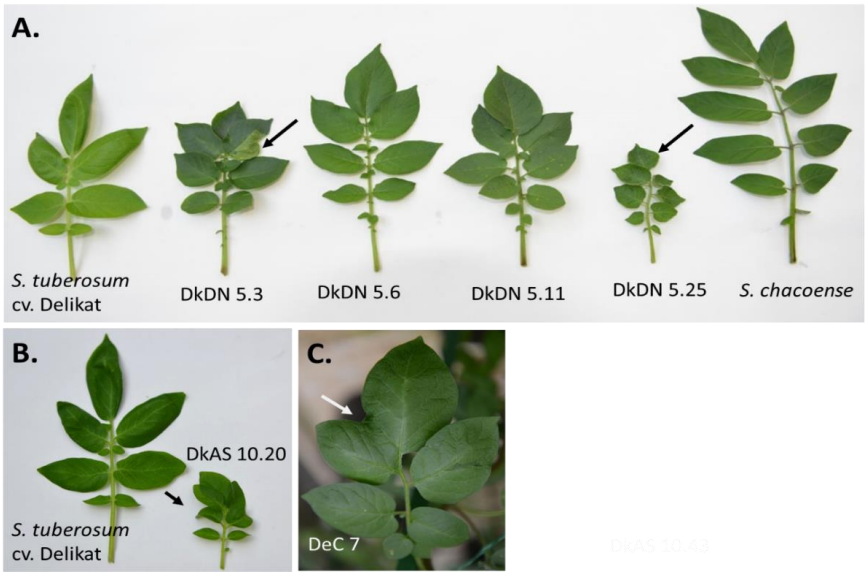

Figure 2. Comparison of the phenotypes of the somatic hybrids with potato parent cv. Delikat, and *S. chacoense*. **(A)** Phenotypes of the plants grown in a greenhouse (61 days), from left to right: potato cv. Delikat, SHs with MMR deficiency (DkDN 5.3, 5.6, 5.11, and the mutator phenotype of DkDN 5.25) and the wild type *S. chacoense*. **(B)** Leaf morphology of the mutator phenotype DkAS 10.20, which shows dwarf phenotype and small leaf with round folioles in comparison with the parent plant cv. Delikat. **(C)** Conjoint leaf of the SH DeC 7 with a proficient MMR system.

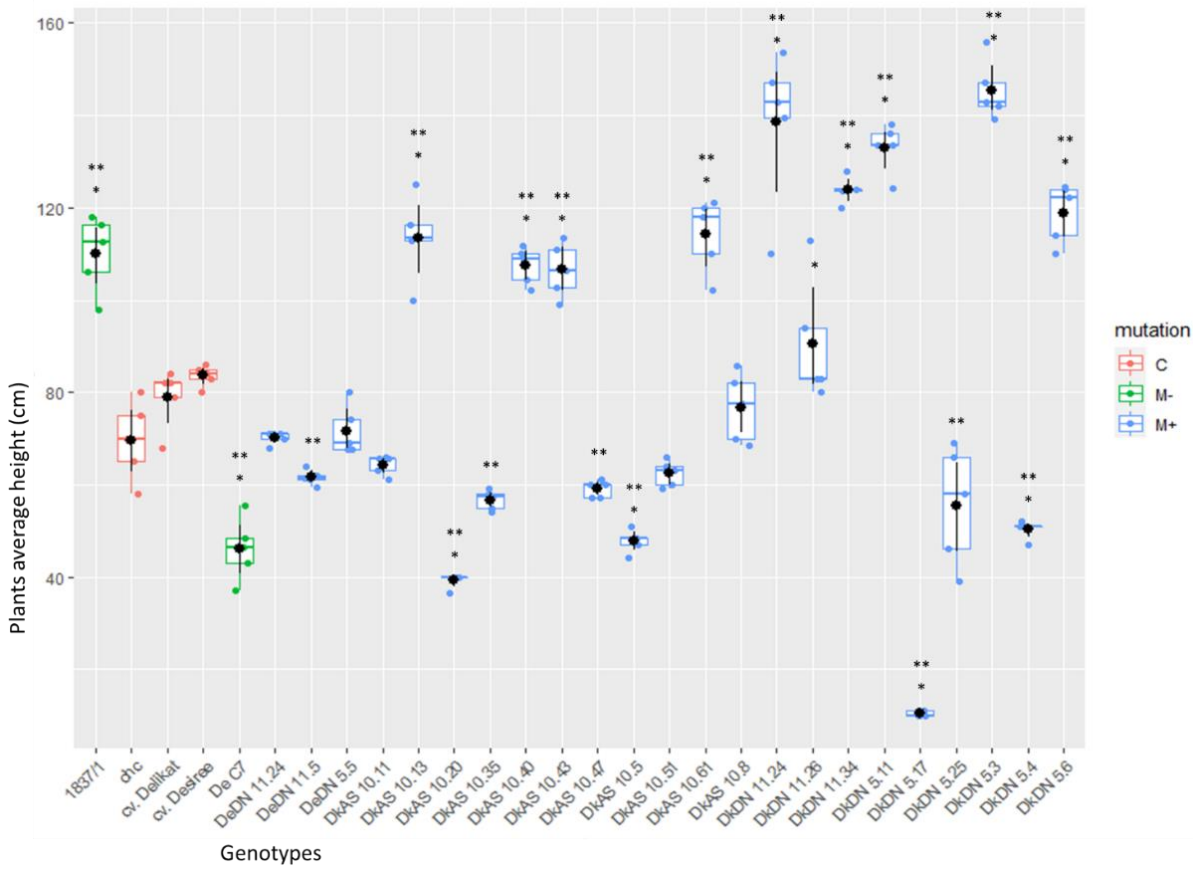

Figure 3. Average height (in cm) of the somatic hybrids of potato (cv. Delikat or cv. Desiree) + *Solanum chacoense*, with or without MMR deficiency, grown in a greenhouse for 61 days after transfer from *in vitro*. Box plots represent means, dots are outliers, the horizontal line in the box represents the median, and the vertical line in the box represents the SD. Pairwise t-test, $p < 0.05$. * Significantly different from *S. chacoense*, ** significantly different from potato cv. Delikat or cv. Desiree, * and ** significantly different from the wt parent plants.

4.1.2. Presence of the *NPTII* selection marker in the MMR deficient transgenic lines

The construct, which was used to generate transgenic *S. chacoense* plants using the antisense or the dominant-negative *AtMSH2* gene, contained as selection marker the neomycin phosphotransferase gene (*NPTII* gene). In the presence of this selection marker, the transgenic plants can grow normally and grow roots on medium containing antibiotics such as kanamycin. All of the SHs with a deficiency in the MMR system, which shows MSI, developed roots on this selection medium, except DkDN 11.10 and DkDN 11.26 (

Table 1). It is possible that after subsequent micropropagation, these two genotypes have loosed the transgene.

Transgene inactivation was reported in plants (Broer, 1996). Nevertheless, heat treatment in transgenic *Medicago sativa* has driven to an almost complete (95%) loss of the phosphinothricin resistance (Walter et al., 1992). However, the loss of the transgene increased the possibility of these genotypes being introduced into breeding programmes and gaining consumer acceptance.

4.1.3. Microsatellite analysis in potato somatic hybrids

MMR deficiency is strongly correlated to the instability of the microsatellites (MSI). MMR deficient plants show MSI, such as deficiency in the *MSH2* gene (Leonard et al., 2003; Hoffman et al., 2004; Depeiges et al., 2005; van Marcke and Angenon, 2013; Rakosy-Tican et al., 2019), *PMS1* gene (Xu et al., 2012), *PMS2* gene (Chao et al., 2005), *MSH6* gene (Jiang et al., 2020). Microsatellite instability is a result of replication slippage or stalling, which occurs when the MMR system is deficient and is also hotspots of chromosomal double-strand breaks (DSBs) (Gadgil et al., 2017).

Chromosome-specific SSR markers (96 in total, 12 with an unknown chromosome location) were used to analyse MSI in SHs and wild type parents. In the somatic hybrids, low polymorphism was observed, and only six SSR markers show MSI as follows: StI0001, StI0027, StI0046, StI0054, STM0024 whereas STG0001 shows MSI also in SHs backcrossed lines with proficient MMR system.

Instability in microsatellite repeated motifs and transcript variations in different MMR genes was reported in hybrids between rice and wild rice (Dong et al., 2013). This indicates a strong relationship between MMR genes and genomic variation, which they cause. We have found microsatellite instability on chromosomes 4, 8, 11, and 12, as soon as the other microsatellites do not show MSI.

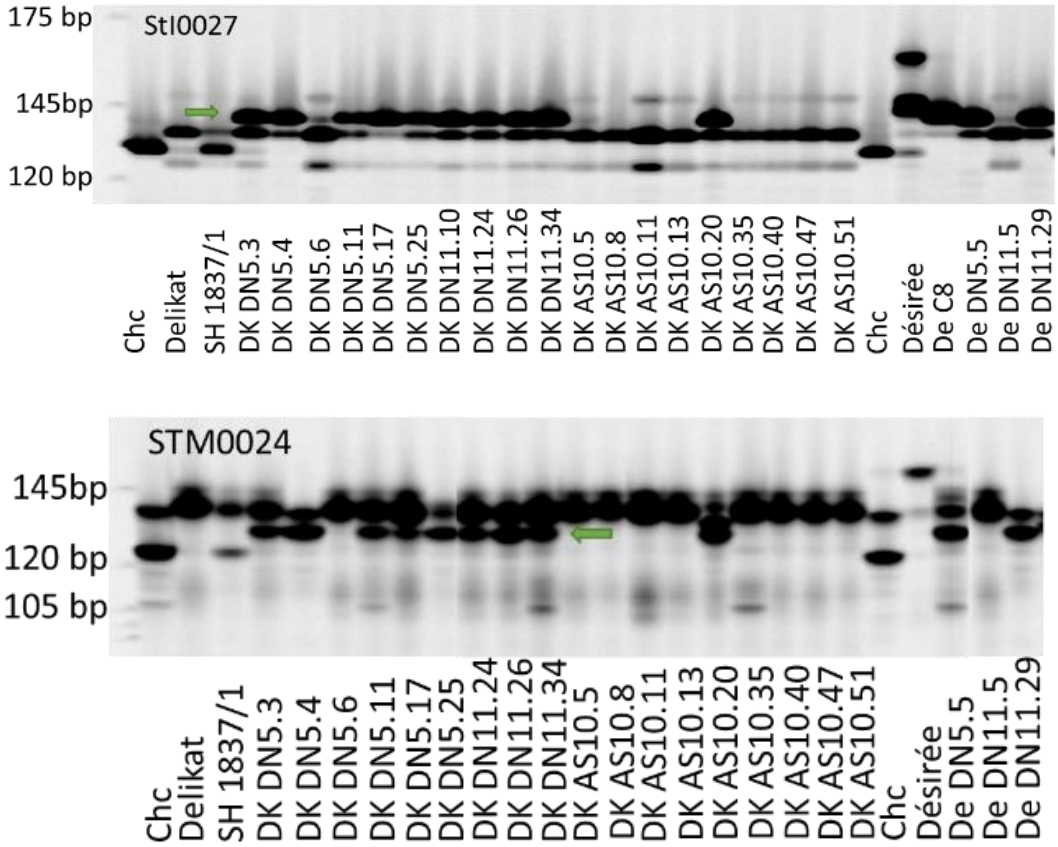

Figure 4. SSR instability in *S. tuberosum* + *S. chacoense* MMR deficient somatic hybrids in comparison with their parents or MMR proficient SH 1837/1 and De C8. Microsatellite marker StI0027 and STM0024. The specific bands that show the SSR instability (MSI) are indicated with a green arrow.

These varied microsatellites included one di-nucleotide-repeat, four tri-nucleotide repeats, and one motif (STM0024) which has five tri-nucleotide repeats and 18 di-nucleotide repeats, accounting for 3% (1/33), 8% (4/50), and 1% (1/96), respectively. The instability appears not only in the increasing number of bands, but also the displacement of the main band position occurs in STM0024 and StI0027 (*Figure 4*). SHs with MMR deficiency: DkDN: 5.3, 5.4, 5.11, 5.17, 5.25, 11.10, 11.24, 11.34, DeDN: 5.5, 11.29, and DkAS 10.20 present MSI with all six SSR markers, on the chromosomes 4, 8, 11, and 12.

4.1.4. The relative transcript level of the *MSH2* and *SPO11* genes in somatic hybrids

The somatic hybrids with a deficiency in the MMR systems were analysed for the accumulation of the *MSH2* and *SPO11* transcript in vegetative and generative tissue. As a control, the transgenic *S. chacoense*, HLDN 5, was used. In *Figure 5*, the relative expression of *MSH2* gene in leaves (**A**) and floral buds (**B**), fluctuate between the samples compared to the MMR deficient *S. chacoense* HLDN 5, used as control. Nevertheless, one MMR deficient somatic hybrid, DkDN 5.3, shows

reduced *MSH2* expression in leaves compared to the MMR deficient *S. chacoense* (p=0.001, *Figure 5- A*). Both wild type (wt) parents, *S. tuberosum*, and *S. chacoense (S. chc)* of the somatic hybrids show a high expression of the *MSH2* gene in leaves, in comparison with the MMR deficient *S. chacoense* (HLDN 5).

This data indicates that the DN mutation induced in the *MSH2* gene can reduce the transcript level of the *MSH2* gene in somatic hybrids. Although the DkAS 10.20 SH shows an increase in the transcript level, this could be a result of the genetic instability, which is revealed by the mixoploidy (Rakosy-Tican et al., 2019).

In general, in the floral buds, the expression level of the *MSH2* gene shows a decrease in comparison to the expression from the leaves. This data corresponds to the data described for tomato, where the highest expression of the *MSH2* was observed in the leaves followed by a slightly lower expression in the floral buds (Tam et al., 2009).

In the floral buds, DkDN 5.11 MMR deficient line is downregulated (p = 0.008). Besides this, the genotype DkDN 5.25 shows a near significant downregulation of the *MSH2* gene (0.52) compared to the transgenic *S. chacoense* (p = 0.07, *Figure 5-B*). The wild type of parental lines, cv. Delikat and *S. chacoense* are slightly downregulated in floral buds while in the leaves are up-regulated. Moreover, *S. chacoense* is significantly up-regulated in leaves (p = 0.0001), in the floral buds is near significantly under-regulated (p = 0.01) in comparison with the MMR deficient *S. chacoense*. Besides this, the tetraploid potato, cv. Delikat is significantly downregulated in floral buds with relative expression of 0.28 (p = 0.001, *Figure 5-B*).

After the silencing of the *MSH2* gene in *N. tabacum* and *N. plumbaginifolia*, the *MSH2* transcript level was assessed using RTqPCR. Here as well, a high variation in the transcript level in leaves was observed, and no one genotype reached zero expression. Moreover, the reduction of *MSH2* transcript level with 20-30% was enough to induce mutator phenotypes such as chimeric albino plants and herbicide tolerance (van Marcke and Angenon, 2013).

This could be the effect of the dominant-negative mutation of the *MSH2* gene in SHs; however, the somatic hybrids are not completely deficient in the *MSH2* gene because the tetraploid potato parent, cv. Delikat is a wild type plant. Nevertheless, this semi deficiency is enough to induce mutations in the genome of the SHs and to increase homeologous recombination between homeologous sequences and subsequent the formation of the double-strand breaks (DSBs), which are a requirement for meiotic recombination.

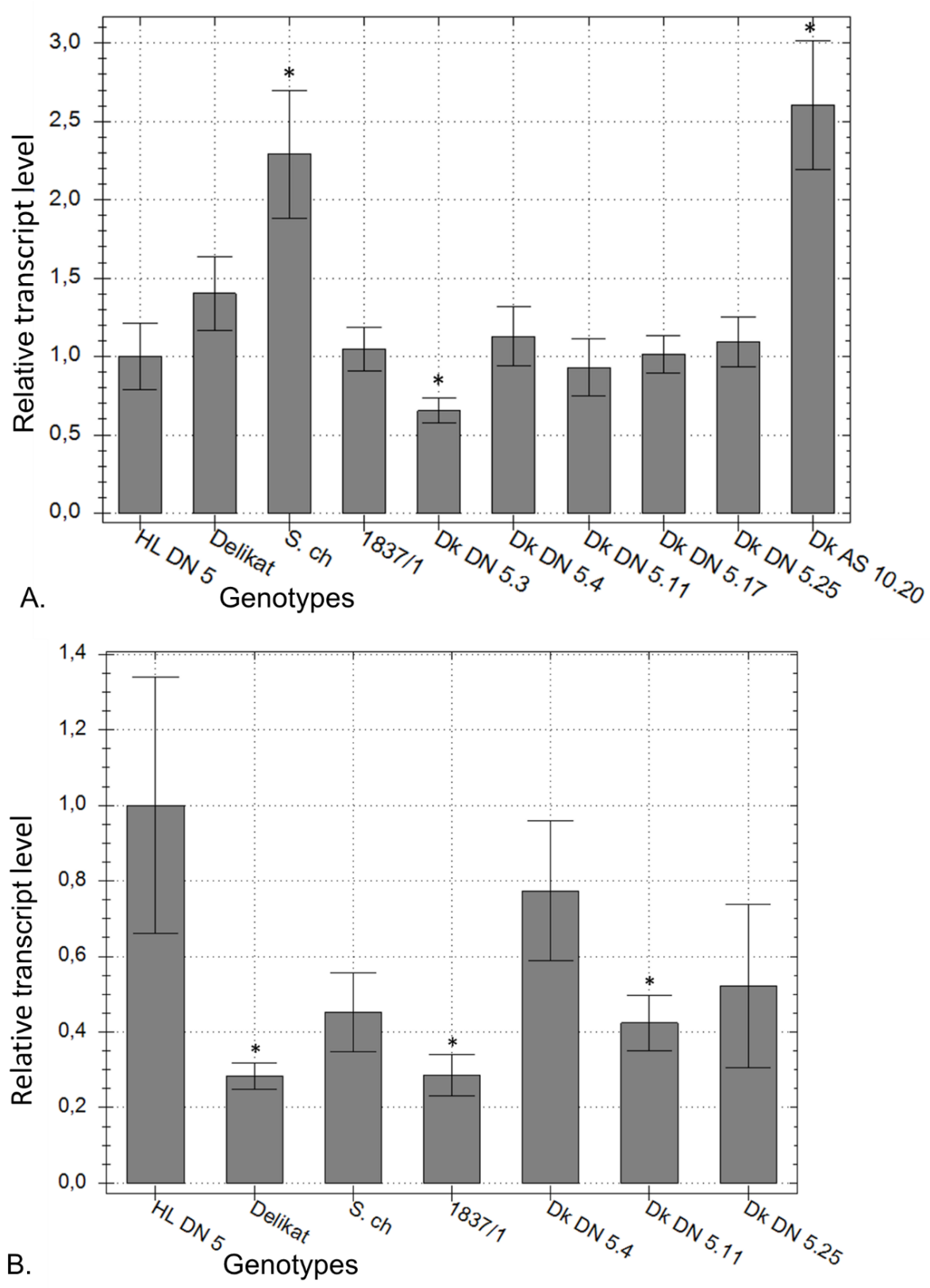

Figure 5. Quantitative real-time RTqPCR analysis of the *MSH2* gene in potato somatic hybrids with and without MMR deficiency in comparison with HLDN 5 - high leptine producer *Solanum chacoense* with a deficiency in the *MSH2* gene (MMR deficient) and the wild type potatoes *S. chacoense* and *S. tuberosum* cv Delikat. **(A)** RTqPCR in leaves, **(B)** RTqPCR in floral buds; Bars represent the standard error of the mean of the replicates (± SEM). * Significantly different from HLDN 5 ($p < 0.05$).

The relative expression of both *SPO11* genes (*SPO11-1* and *SPO11-2*) was analysed because they are essential for DSB formation in plants but how they act together in DSB formation is still unclear (Grelon et al., 2001; Hartung et al., 2007; Sprink and Hartung, 2014). No one from the SHs with a mutator phenotype shows a downregulation in *SPO11* expression.

Moreover, our data show that the MSH2 protein is required in the repair of the DSBs because neither the *MSH2* nor the *SPO11* genes are under-expressed in the floral buds in the case of the SHs with MMR deficiency. An exception of this is the DkDN 5.11 hybrid, where the *MSH2* transcript is downregulated while the *SPO11* gene is slightly up-regulated in comparison with the control, but this increase is not statistically relevant.

Our data suggest a co-regulation of the *MSH2* and *SPO11* genes in floral buds, which confirms the hypothesis that *MSH2* plays an essential role in meiotic recombination. Several studies indicate the function of *MSH2* in meiosis (Meyer et al., 2001; Lloyd et al., 2007; Tam et al., 2011; Manhart and Alani, 2016; Sarma et al., 2018).

A co-regulation between SPO11 and MSH2 was already described in *S. cerevisiae* (Meyer et al., 2001). Moreover, a regulatory cis-element except a base pair is identical to the 5` flanking region sequence of SPO11, deletion of this element causes the loss of meiotic induction of MSH2 (Meyer et al., 2001). In mice ovary cell lines, it was observed that the MSH2 protein plays a role in NHEJ type DSB repair, impeding the joining of the mismatched DNA termini (Smith et al., 2005). Besides this, another reported study supports the role of the MMR proteins in DSBs repair in mouse fibroblasts, where MLH1 inhibits the repairing of DSBs, which contains noncomplementary base pairs (Bannister et al., 2004).

4.1.5. Genomic *in situ* hybridization of potato somatic hybrids in meiosis

Genomic *in situ* hybridization (GISH) is a modification of fluorescent *in situ* hybridization (FISH), which allows distinguishing genomes from different species in a cell and allows the identification of parental chromosomes. *Solanum chacoense* Bitt. is a very close relative of cultivated potato, *S. tuberosum*. Both are species classified in section Petota, 4th clade (Spooner et al., 2018).

S. tuberosum (2n=4x=48) has an AAAA genome, while *S. chacoense* (2n=2x=24) has an AA genome. Therefore, their genome is very homolog, and a genome differentiation using standard GISH conditions is not possible. Genomes, which have 80-85% homology, can be differentiated using standard conditions. On the other hand, for the genomes, which share 90-95% homology, the conditions for GISH must be improved (Silva and Souza, 2013). Such improvement consists

of an increase of the blocking DNA and the avoidance to use formamide (Parokonny et al., 1997; Jang and Weiss-Schneeweiss, 2015). Such optimizations were pivotal to differentiate between the genome of *S. tuberosum* and *S. chacoense* in SHs with MMR deficiency. This study aimed to evidence the homeologous recombination or introgression of *S. chacoense* in SHs with MMR deficiency in early meiosis stages.

It is well known that MMR proteins suppress recombination between two divergent sequences since the meiotic recombination in plants is based on DNA homology (Bozza and Pawlowski, 2008).

For GISH analysis, we have chosen the SH DkDN 5.4 with MMR deficiency, which shows MSI, mutator phenotype, and strong resistance to CPB (*Figure 4*,

Table 1).

DkDN 5.4 possesses strong toxicity and deterrent effect toward CPB, such as the parent plant *S. chacoense*, this evidence the introgression of this trait into the gene pool of this new hybrid. Chromosome specific SSR analysis shows the inheritance of some chromosome-specific alleles from *S. chacoense* and potato (Figure 4).

Using GISH analyses in male meiocytes, it was possible to identify in SH DkDN 5.4, in the early meiosis (*Figure 6*), chromosome regions from *S. chacoense*, which forms chiasma with chromosomes from *S. tuberosum.* In male meiosis of this SH, many meiotic aberrations were observed, such as the formation of univalents in diakinesis and aberrant chromosome number. Consistent with this, the anthers of this SH contained shrunken pollen at the end of the meiosis.

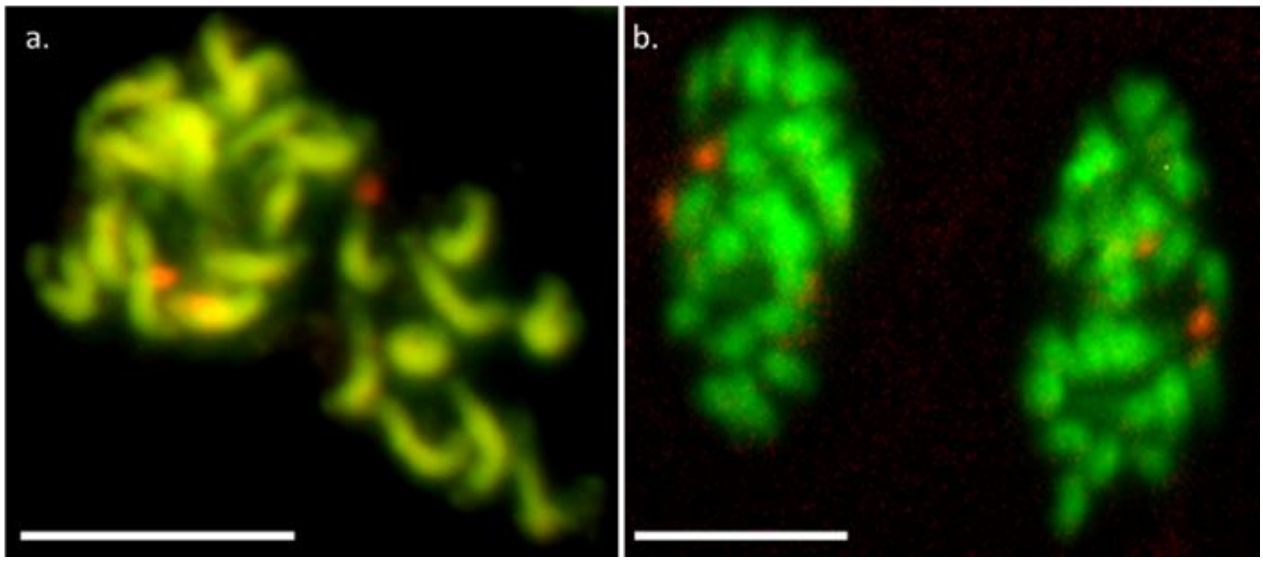

Figure 6. Male meiotic chromosomes in DKDN 5.4 SH; **(a)** diakinesis, **(b)** anaphase I; red - *S. chacoense*; green - *S. tuberosum*; bars 10 μm.

Similar observations were made in the diploid tomato, *MSH2* silenced lines where the majority of meiocytes showed tetraploid meiocytes and abnormal tetraploid meiocytes. Just a few meiocytes showed normal diploid meiosis. Besides this, fertility was affected, and the *MSH2* silenced line showed reduced pollen viability, which has affected seed production as well (Sarma et al., 2018).

Moreover, the analysed SHs with MMR deficiency show reduced pollen viability, which was significantly lower ($p < 0.05$) compared to wild type plants. Sterility is a common trait caused by a deficient MMR system as it was observed in other *MSH2* deficient plants (Hoffman et al., 2004; Sarma et al., 2018).

Our results are similar to these findings. Therefore, the observed aberrant meiosis and very low pollen viability are most probably co-induced by somatic hybridization and low MSH2 protein activity. MSH2 increases significantly homeologous recombination. The aberrant meiosis is a cause of deficient heterodimer formation with MSH7.

From the best of our knowledge, until now, it was not reported discrimination between two tuber-bearing A- genomes, except SHs between *S. bulbocastanum* (diploid, 1EBN) and *S. tuberosum* (Iovene et al., 2007; Rakosy-Tican et al., 2020).

Overcoming hybridization barriers and introgressing resistance genes from wild relatives are at very high importance for potato breeding. Therefore, tracking the introgression of parental chromosomes in the new genotype is very important.

4.1.6. MMR deficiency increases the antibiosis and antixenosis towards CPB in MMR deficient SHs

S. chacoense is a rich reservoir of resistance genes, including resistance to Colorado potato beetle (CPB) (Sinden et al., 1986; Brown and Thomas, 1994; Hawkes, 1994).

The resistance to CPB is given by the ability of this wild plant to produce steroidal glycoalkaloids (SGA), which are known as leptines (Sinden et al., 1986; Brown and Thomas, 1994; Hawkes, 1994).

Leptines have anticholinesterase-type activity; therefore, one nanomole concentration is enough to have a deterrent and toxic effect for CPB (Sinden et al., 1980; Sinden et al., 1986; Ronning et al., 1999; Rangarajan et al., 2000; Yencho et al., 2000; Dinkins and Peterson, 2008). Leptines are

synthesized only in leaf tissue, absent in tubers, and do not affect tuber quality; this trait is essential for the market and consumption (Veilleux and Miller, 1998).

To prove the resistance of these hybrids toward CPB, an antibiosis and antixenosis assay were done. It was found that the SHs with MMR deficiency show good resistance to CPB, and therefore, the introgression of this trait was successful (

Table 1, Supplement).

The data from this chapter is published in:

Molnár, I., **Besenyei, E.**, Thieme, R., Thieme, T., Aurori, A., Baricz, A., et al. (2017). Mismatch repair deficiency increases the transfer of antibiosis and antixenosis properties against Colorado potato beetle in somatic hybrids of Solanum tuberosum + S. chacoense. *Pest management science* 73, 1428–1437. doi: 10.1002/ps.4473.

4.2. Inducing mutations in the potato *MSH2* gene with CRISPR/Cas9

4.2.1. *In vitro* cleavage assay

We designed several sgRNAs for the *MSH2* gene using the CCTop-CRISPR/Cas9 target online predictor to predict the possible off-target effects (Stemmer et al., 2015; Labuhn et al., 2018). The sgRNAs with lower possible off-target effects were used to assess the cleavage of each sgRNAs using an *in vitro* cleavage assay. All sgRNAs used in this study were designed for the protospacer to correspond to 20 nt target site in the *MSH2* gene and to create DSBs at 3 bp upstream of the protospacer adjacent (PAM) motif. To target the *MSH2* gene, which is located on chromosome six on potato, we have chosen six sgRNAs for the *in vitro* cleavage assay (*Figure 7*). The sgRNA2 is located on exon 3, sgRNA5 and sgRNAs10 are located on exon 4, and sgRNA22 is located on exon 5 (*Figure 7*).

The last two designed sgRNAs, sgRNA32, and sgRNA38 are located on exons 12 and 13, respectively (**Figure 7**).

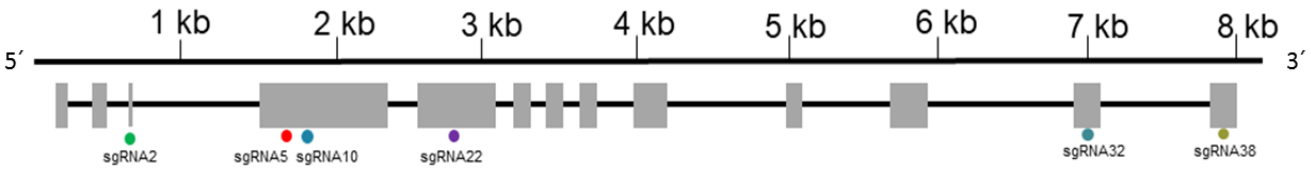

Figure 7. Schematic representation of the *MSH2* gene from potato, gray lines are the exon sequences, and black lines are the introns. The dots represent the localization of sgRNAs used in the experiments.

All tested sgRNAs were able to cleave the target PCR product of the *MSH2* gene from potato and show different cleavage efficiency *in vitro*. The sgRNA2 and sgRNA38 show the highest efficiency and were therefore chosen in subsequent experiments for the transfection of protoplasts and *Agrobacterium tumefaciens* mediated transformation in potato (*Figure 8*).

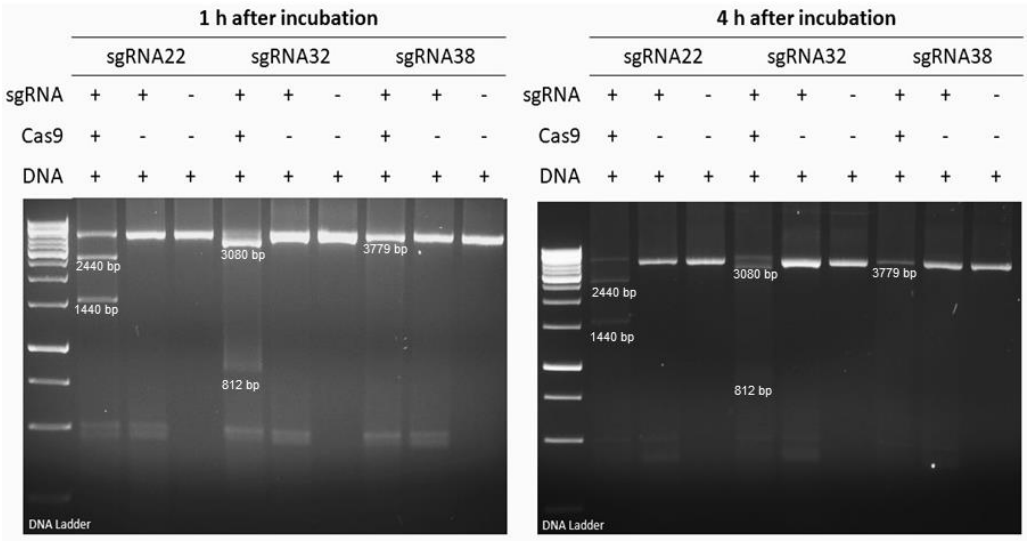

Figure 8. In vitro testing of the sgRNAs after 1 h and 4 h incubation time with Cas9 and different sgRNAs.

4.2.2. DNA-free genome editing in potato protoplasts

Genetic engineering is an efficient way to introduce important agronomical traits for potato improvement. Inducing mutations in the target genes using *in vitro* synthesized sgRNAs, preassembled with the Cas9 endonuclease, which together forms the ribonucleoprotein complex (RNPs), is beneficial because the stable integration of foreign DNA in the genome is avoided. This is very important because of the legislative regulation perspective. Some countries tend to a

process-based regulatory system such as Europe, Australia, New Zeland, and India (Friedrichs et al., 2019).

For plant breeding, DNA-free genome editing is a very recent method which still needs optimization to increase the efficiency and specificity. Therefore, DNA-free genome editing requires the optimization of regeneration of recalcitrant crops. Using CRISPR/Cas9 ribonucleoproteins (RNPs), we have targeted the potato *MSH2* gene. This gene plays an essential role in DNA recombination and repair. To achieve this goal, we have isolated potato mesophyll protoplasts and made transfections using RNPs. For isolation and regeneration, we have used an already established protocol (Thieme et al., 2008).

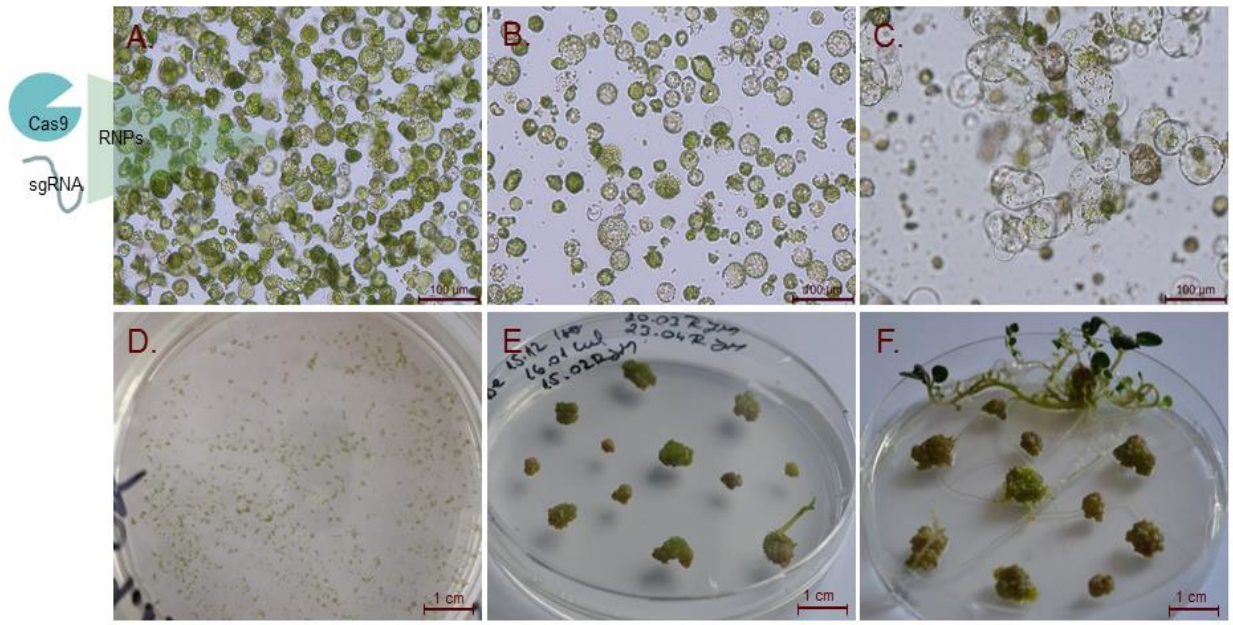

Figure 9. Schematic representation of the direct delivery of RNPs (Cas9 and sgRNA) to potato protoplasts to produce transgene-free edited crop plants. **(A)** Freshly isolated protoplasts are transfected with RNPs using PEG. **(B)** Freshly transformed potato protoplasts are transferred to the regeneration medium. **(C)** Protoplasts two weeks after transfection, the cell wall is restored. **(D)** Protoplasts four weeks after transfection **(E)** Protoplasts 4 months after isolation **(F)** Shoot regeneration 5 months after protoplast isolation.

However, the regeneration of potato protoplasts after PEG transfection with RNPs was not successful, although the regeneration of potato protoplasts without using RNPs worked very well (*Figure 9; E-F*). DNA-free genome editing in potato with RNPs *via* PEG transfection was reported as successful by other research groups by using another isolation and regeneration method involving the embedding of protoplasts in alginate, after the PEG transfection (Andersson et al., 2017; González et al., 2020). After the transfection of potato protoplast, using sgRNA2 and

sgRNA38, the DNA from the protoplasts was isolated, and the target regions were sequenced. Different mutations 3 nucleotides upstream the PAM sequence were observed.

In conclusion, the PEG transfection method was successful, but the plant regeneration require optimization to obtain regenerated mutants. Nevertheless, using a liquid medium for protoplast regeneration is a more convenient method because, in every step, other interventions could be performed without disturbing them, which would not be the case if they would be embedded in alginate.

4.2.3. *Agrobacterium*-mediated transformation in potato

In this study, we have used the *Agrobacterium*-mediated transformation, using the pDECas9 T-DNA binary vector (Fauser et al., 2014) to deliver the Cas9 endonuclease and the sgRNA. Once inside the plant cell, the components of the T-DNA binary vector are expressed, and the T-DNA integrates into the plant genome resulting in stably transformed potato lines.

Using *Agrobacterium tumefaciens* GV3101-pMP90RK strain and the pDECas9 binary vector with the sgRNA targeting the exon 3 and 13 from the *MSH2* gene, we were able to obtain five stable transgenic lines with the sgRNA2. By using the sgRNA38, no plant regeneration occured, the callis do not regenerate shoots, and however, they developed normally in the first stages.

For the transformation experiment, we used potato internodes in which plant regeneration was successfully achieved.

The five regenerated transgenic potato lines targeting the exon 3 (DksgRNA2), were sequenced to reveal if mutations occur in the target sequence. In two, potato lines modifications in the target sequence were observed; in the other three lines, no mutation was detected in the targeted region. In the exon 3 a three-nucleotide deletion was observed three nucleotides upstream the PAM motif, which changes the open reading frame (ORF) of the *MSH2* gene (*Figure 10*). In the second line, a substitution was observed as well as in the exon 13 using the sgRNA38 (*Figure 10*). It was not possible to regenerate plants after the co-culture with A. *tumefaciens*, which contained the transgene for sgRNA38 to knock-out the exon 13. The obtained callus has died before shoots appear. However, from a rescued callus, DNA was isolated and sequenced in the target region, and a substitution was found. This substitution changes the frameshift of the MSH2 protein in a strong conserved C-terminal region (*Figure 7*), which is an ATP/ADP binding domain.

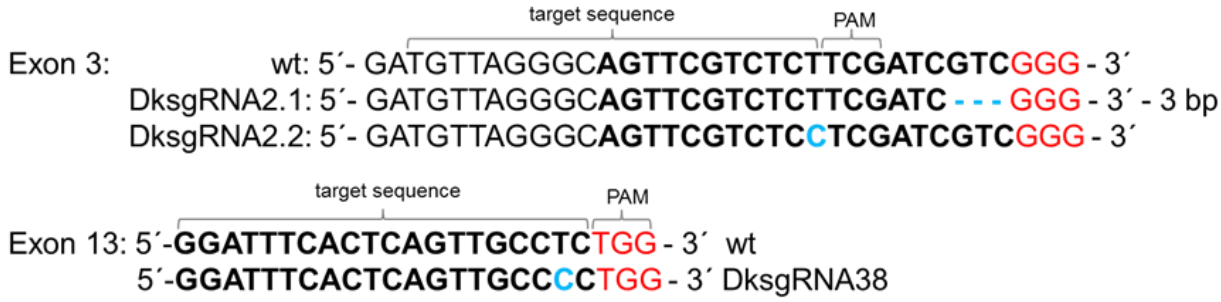

Figure 10. Mutations in the regenerated genotypes obtained after *A. tumefaciens* transformation, determined by DNA sequencing in the exon 3 (DksgRNA2) and 13 (DksgRNA38) of the *MSH2* gene.

This ATP/ADP binding domain is required for mismatch correction and the mismatch binding (Bowers et al., 1999; Dufner et al., 2000). Moreover, a mutation in the ATP/ADP binding domain in mice was associated with tumor proliferation (Lin et al., 2004). The MutS-DNA complex formation occurs in an ATP dependent manner (Iyer et al., 2006). It is possible that in plants, losing the ATP binding domain of this protein is not compatible with life. This could explain the impossibility to regenerate plants with a mutation in this domain.

During the regeneration, mutant phenotypes were observed, which correlate with mutations in the exon 3 (DksgRNA2). They consist of deformed and albino shoots (*Figure 11*). From the mutant phenotypes, it was not possible to regenerate viable plants.

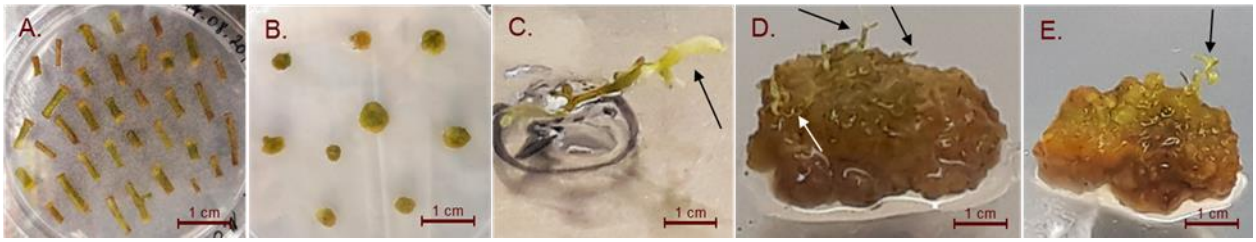

Figure 11. Agrobacterium-mediated CRISPR/Cas9 genome editing in potato, targeting the *MSH2* MMR gene in exon 3 **(A)** callus induction in potato, **(B)** callus regeneration, **(C, D, E)** potato mutator phenotypes obtained during the regeneration.

The suppression of the *MSH2* gene leads to an increased mutation frequency in the genome, consisting of the appearance of small insertions and deletions (indels) and single-nucleotide variant mutations. This could explain the occurrence of potato mutant phenotype during the regeneration process (Belfield et al., 2018). The chimeric albino phenotype was observed in genome-edited potato plants, where the apical meristem was completely white without true leaves (*Figure 11; C-D*), and white leaves were observed (*Figure 11- E*). This data corresponds with the observations

made in *N. tabacum* and *N. plumbaginifolia* where the *MSH2* gene was silenced. In this case, large white sectors on the first pair of leaves were observed, which appears more rarely in the cotyledons (van Marcke and Angenon, 2013).

Moreover, in the moss *Physcomitrella patens*, which has a mutation in the *MSH2* gene, pleiotropic growth, and developmental defects were observed, such as undeveloped buds, shoots without leaves, sterile phenotype and decreased cell division (Trouiller et al., 2006).

The *A. tumefaciens* mediated genome editing is the most convenient and routinely used technique for genome editing in many species. However, in potato is challenging because of the stable integration of the transgenes and clonally propagation. For transgene elimination, selfing is applied, but many potato cultivars are self-incompatible. The development of a routine DNA-free genome editing technique is crucial for potato. In this study, we could show that genome editing using *A. tumefaciens* and the DNA-free genome editing are both reliable methods for potato transformation. However, the DNA-free genome editing presents an advantage over the *Agrobacterium*-mediated transformation, but further optimizations of plant regeneration are needed.

Chapter 5. General conclusions

We have demonstrated the transcriptional levels of the *MSH2* gene from the SHs in vegetative and generative tissues. The transcription level in SHs with MMR deficiency was much lower than the wild type plants, which can account for the MMR deficiency. Furthermore, an exception was found in the SHs generated with the antisense construct (DkAS 10.20), which shows a high transcript level in the leaves. The high transcription level could be the result of the genetic instability of this plant, which is mixoploid.

In the buds of the SHs, it has been shown that the DSB initiator SPO11 proteins are co-regulated with MSH2, indicating the role of MSH2 in meiosis.

Nevertheless, we have proved that this half-deficiency is sufficient to induce mutations in the genome of the SHs and to increase homeologous recombination between homeologous sequences, and subsequently, the formation of the DSBs, which are a prerequisite for meiotic recombination.

The recombination between the *S. chacoense* and *S. tuberosum* was shown in early meiosis for the DkDN 5.4 SH, where a Y-type chromosome appears, indicating the chiasmata formation between heterologous chromosomes. Altered meiosis and low pollen viability were also observed. All of these emerge as patterns of MMR deficiency, and these data correspond with those reported in the literature.

Testing the SHs seven years after they were produced for the transgene integration, revealed that 13 out of the 22 tested SHs had lost the transgene (*NPTII*). This is a very useful feature because these plants could be used as pre-breeding material. For example, the DkDN 11.10 hybrid possesses resistance to CPB, but it no longer has the transgene.

The main objective in creating these SHs was to introduce resistance to CPB from *S. chacoense*. It was shown that the SHs with MMR deficiency are more resistant to CPB than proficient MMR

SHs and some of their progenies (Molnár et al., 2017). In addition, one SH (DkDN 5.4) exhibited strong resistance to CPB, similar to *S. chacoense*.

Phenotypic analysis of the SHs with MMR deficiency revealed a high frequency of various mutant phenotypes: dwarf plants, small conjoint leaves, early flowering, no flower formation, and low pollen viability, which cannot be explained by their hybrid nature alone. The deficient MMR system causes these phenotypes, and our data correspond with the data shown in other MMR-deficient plants. EMS mutagenesis is highly useful and extensively applied in breeding, but it only causes transitions from G:C to A:T (Colbert et al., 2001; Greene et al., 2003; Till et al., 2003), while MMR deficiency affects the encoded genetic regions (Belfield et al., 2018). Therefore, MMR deficiency is more beneficial for breeding as EMS, but the acceptance of transgenic plants is shallow.

With a DNA-free genome editing method, these limitations could be overcome, at least in those countries that do not follow a process-based genome editing regulation.

Here we have shown an efficient method for DNA-free genome editing, as well as stable transformation with CRISPR/Cas9. Using *A. tumefaciens* for delivery of the CRISPR/Cas9 system, we were able to partially inactivate the *MSH2* gene, obtaining potato plants with the mutator phenotype

We were able to show deletions 3 nt upstream the PAM region in the *MSH2* gene with DNA-free genome editing method and with *Agrobacterium*-mediated genome editing. After the delivery of the CRISPR/Cas9 system by *A. tumefaciens*, the regenerated shoots showed the albino phenotype and shoots without true leaves. In the DksgRNA2.1 line, which showed no phenotypic alterations (***Fehler! Verweisquelle konnte nicht gefunden werden.***, supplementary materials), the relative gene expression was analysed and showed a reduction in the *MSH2* level compared to the wild type plant.

Further optimizations are needed in order to increase the efficiency of transformed plants and plant regeneration from potato protoplasts.

DNA-free genome editing offers an excellent perspective for increasing resistance in potato to biotic and abiotic stress associated with monogenic traits. Considering the polygenic traits, we foresee the usefulness of inducing MMR deficiency for acquiring a necessary genetic variability in targeted plants, by increasing the rate of mutations and homeologous recombination.

The results from this thesis show that manipulating the MMR system is a promising tool for plant breeding as well as genome editing to increase potato resistance to different diseases.

Overall, it was possible to show patterns of the MMR deficiency in both SHs and genome edited plants, and it was possible to identify SHs with resistance to CPB and to show the recombination between homeologous chromosomes.

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Supplementary materials

Table 1. Summary of ploidy, presence of the *NPTII* transgene, antibiosis, antixenosis and MSI in the somatic hybrids and their parents.

Somatic hybrids or parents	Clone	Ploidy	Presence of the *NPTII* gene	MSI	Antibiosis	Antixenosis
***S. tuberosum* cv Desiree**	Parent	4x	-	-	-	-
***S. tuberosum* cv Delikat**	Parent	4x	-	-	-	-
***S. chacoense* HL**	Parent	2x	-	-	++	++
***S. chacoense* HL AS 10**	Transgenic line	4x	ND	-	ND	ND
***S. chacoense* HL DN 5**	Transgenic line	2x	ND	-	ND	ND
***S. chacoense* HL DN 11**	Transgenic line	2x	ND	ND	ND	ND
cv Delikat + *S. chacoense* HL AS 10	DKAS 10.5	4 x	-	-	-	-
	DKAS 10.8	4x	-	-	ND	ND
	DKAS 10.11	4x mixo	-	-	-	-
	DKAS 10.13	4x	ND	-	+	+
	DKAS 10.20	4x-6x mixo	+	+	-	-
	DKAS 10.35	5x	-	-	+	-
	DKAS 10.40	4x	-	-	+	+
	DKAS 10.43	4x	-	ND	+	+

	DKAS 10.47	4x	-	-	+	+
	DKAS 10.51	4x	ND	-	-	-
	DKAS 10.61	4x	-	ND	+	+
cv Delikat + *S. chacoense* HL DN 5	DKDN 5.3	6x	+	+	-	-
	DKDN 5.4	Nd	+	+	++	++
	DKDN 5.6	4x	-	-	-	-
	DKDN 5.7	4x-6x mixo	-	ND	++	++
	DKDN 5.11	6x	+	+	+	+
	DKDN 5.17	6x	+	+	-	-
	DKDN 5.25	6x-8x mixo	+	+	ND	ND
cv Delikat + *S. chacoense* HL DN 11	DKDN 11.10	4x-6x mixo	-	+	+	-
	DKDN 11.24	6x	+	+	-	-
	DKDN 11.26	7x	-	+	ND	ND
	DKDN 11.34	5x	+	+	+	+
cv Desiree + *S. chacoense* HL DN 5	DeDN 5.5	4x	+	+	+	+
cv Desiree + *S. chacoense* HL DN 11	DeDN 11.5	4x	ND	-	ND	ND
	DeDN 11.24	6x	ND	ND	ND	ND
	DeDN 11.29	Nd	ND	+	++	+
cv Desiree + *S. chacoense* HL	DeC8	5x-6x mixo	-	-	ND	ND

Notes: AS-antisense and DN- dominant-negative *MSH2* gene. ND – not determined, + = yes, - = no.

Table 2. Type of mutation showing mutant phenotype, flowering, pollen viability and MSI in the somatic hybrids between potato + MMR deficient *Solanum chacoense*, showing mutant phenotype in the greenhouse (Rakosy-Tican et al., 2019).

Somatic hybrid	Mutant phenotype	Flowers	Pollen viability %	SSR instability (MSI)
DkDN 5.3	Very small leaves, tall plants, produce stolons as *S. chacoense*	+	**12**	+
DkDN 5.4	Dark purple tubers of variable size, produce stolons as *S. chacoense,* curled leaves	+	**19**	+
DkDN 5.6	Gigantism, large leaves	+	**22**	-
DkDN 5.7	Stunted growth, deformed leaves, early flowering	+	**ND**	**ND**
DkDN 5.11	Large leaves, early flowering	+	**ND**	+
DkDN 5.17	Dwarf, bushy with small leaves, deformed small tubers	-	**ND**	+
DkDN 5.25	Deformed leaves, grey-green color, mutant chloroplasts	+	**ND**	+
DkDN 11.10	Early dehiscent flower, curled leaves	+	**ND**	+
DkDN 11.26	Deformed large leaves	+	**2.4**	+
DkDN 11.34	Gigantic growth,large deep green leaves, early flowering	+	**43**	+
DkAS 10.5	Dwarf plants	+	**ND**	-
DkAS 10.8	Deformed, pale green leaves	-	**ND**	-
DkAS 10.20	Dwarf, bushy, round shape small leaflets	-	**ND**	+
DkAS 10.40	Gigantic leaves	+	**49**	-
DeC 7	Dwarf, large, conjoined leaflets	-	**ND**	**ND**
DeDN 5.5	Stunted growth, large leaves	+	**ND**	+
DeDN 11.5	Tall plants with large leaves	+	**21**	-

Notes: AS-antisense and DN- dominant-negative *MSH2* gene. ND – not determined, + = yes, - = no.

Publications

ISI articles from the results of this thesis:

1. Rakosy-Tican, E. *, **Lörincz-Besenyei, E.***, Molnár, I.*, Thieme, R.*, Hartung, F., Sprink, T., Antonova, O., Famelaer, I., Angenon, G., and Aurori, A.* (2019). New phenotypes of potato co-induced by mismatch repair deficiency and somatic hybridization. *Front Plant Sci* 10, 3. doi: 10.3389/fpls.2019.00003. **IF: 4.402. *equal contribution**
2. Molnár, I., **Besenyei, E.,** Thieme, R., Thieme, T., Aurori, A., Baricz, A., Banciu, H.L., and Rakosy-Tican, E. (2017). Mismatch repair deficiency increases the transfer of antibiosis and antixenosisproperties against Colorado potato beetle in the somatic hybrids *Solanum tuberosum* (+) *S.chacoense*, Pest Management Science, Accepted Author Manuscript. doi:10.1002/ps.4473. ***IF:2.811***

Other ISI articles:

1. Cristea, V., **Besenyei, E**., Jarda, L., Farkas, A., Marcu, D., Clapa, D., Halmagyi, A. and Butiuc-Keul, A. (2019). *In Situ* genetic variability and micropropagation of *Cerastium banaticum* (Rochel) Heuff. (Caryophyllaceae)– a Rare and Endemic Species from Romania, Acta Biologica Cracoviensia Series Botanica 61/1: 53– 62. **IF=1.111**

Article B+ from the results of this thesis:

1. Rakosy-Tican, E., Thieme, R., Aurori, A., Erdelyi-Molnár, I., **Besenyei, E.**, Mustaţă, A.R., Măgineanu, A.M. and Cruceriu D (2016). The application of combinatorial biotechnology in improving potato resistance to biotic and abiotic stress, Studia UBB Biologia, 61(1): 79–88.

Other B+ articles:

1. Marcu, D., **Besenyei E.**, Cristea V., (2014) Radiosensitivity of maize to gamma radiation based on physiological responses, Muzeul Olteniei Craiova. Oltenia. Studii şi comunicări. Ştiinţele Naturii. Tom. 30, No. 1/2014, ISSN 1454-6914.
2. Marcu, D., Halmagyi, A., **Besenyei, E.**, Clapa, D., Fira, A., Cristea, V., (2014) *In vitro* conservation of *Achillea pyrenaica* Sibth. ex Godr., a pyrenean endemic species, Muzeul Olteniei Craiova. Oltenia. Studii şi comunicări. , Ştiinţele Naturii. Tom. 30, No. 2/2014 ISSN 1454-6914 55.

Conference participation from the results of this thesis:

1. **Lörincz-Besenyei, E.**, Metje, J., Hartung, F. and Sprink, T., (2017) DNA-free genome editing in potato, 10th Young Scientist Meeting, Siebeldingen, Germany, November 08-10, 2017, Vol. 192 (08.11.2017), pg.60.
2. Rakosy-Tican, E., Molnar, I., Thieme, R., Aurori, A., **Besenyei, E.**, Denes, T., Thieme, T., Molnar-Lang, M. and Vass, I. (2017) A new potato for a new climate- using different biotechnological tools to improve multiple resistance traits, 20th EAPR Triennial Conference 09.07-14.07.2017, Versailles.
3. **Besenyei, E.,** Sprink, T., Hartung, F. and Rakosy-Tican, E., (2017) Neue Kartoffel Sorten für den Umwelt, DBU Fachkolloquium, Umweltschutz im 21 Jahrhundert, Julius-Kühn Institut, Quedlinburg 4-06.05. 2017.
4. Rakosy-Tican, E., Molnar, I., Thieme, R., Aurori, A., **Besenyei, E.**, Thieme, T. and Vass, I. (2017) New potato crop for a new climate – the application of complex biotechnological tools to improve resistance to biotic and abiotic stress, 2nd Agriculture and Climate Change Conference 26–28 March 2017, Sitges, Spain.
5. **Besenyei, E**., Linc, G. and Rakosy-Tican, E. (2016) Effects of DNA mismatch repair (MMR) system deficiency on homeologous recombination in meiosis, in potato somatic hybrids, Plant Biology Europe EPSO/FESPB 2016 Congress, Prague 26-30 June.
6. Rákosy-Tican, E., Thieme, R., Aurori, A., Molnár, I., Dénes, T.E., **Besenyei, E.**, Marginean, A.M., Cruceriu, D. and Mustata, R.A., (2016) The application of combinatorial biotechnology in improving potato resistance to biotic and abiotic stress, Asociația Română de culturi de țesuturi și celule vegetale (ARCTV), Cluj Napoca, Romania.
7. Molnár, I., **Besenyei, E.**, Thieme, R., Thieme, T. and Rákosy-Tican, E. (2016) Antibiosis and antixenosis properties of somatic hybrids and backcross progenies (*S. chacoense*+ *S. tuberosum*) to the Colorado potato beetle, ARCTV, Cluj-Napoca, Romania.Asociația Română de culturi de țesuturi și celule vegetale, Cluj-Napoca, Romania
8. **Besenyei, E.**, Linc, G. and Rakosy-Tican, E. (2016) Homeológ rekombináció burgonya szomatikus hibridekben a DNS javító rendszer (MMR) gátlásakor, XXII. Növénynemesítési Tudományos Nap, Magyar Tudományos Akadémia (Hungarian Academy of Sciences).
9. **Besenyei E.**, Rákosy-Tican E., Thieme R., Antonova O. and Thieme T., (2015) The analysis of microsatellite instability in MMR deficient somatic hybrids between potato and *Solanum chacoense*, Annual Conference of the German Genetics Society, Book of abstracts p.93, 28-30 September, Kiel.
10. Margineanu A., Molnar I., **Besenyei E.** and Rakosy-Tican E., (2015) Trichome density and Colorado potato beetle choice test, performed in somatic hybrids between two potato cultivars and *Solanum chacoense*, Young Researchers in Biosciences, Cluj-Napoca.
11. **Besenyei E.**, Rákosy-Tican E., Thieme R. and Thieme T. (2015) The analysis of Colorado potato beetle resistance in MMR deficient somatic hybrids between potato and high leptine producer *Solanum chacoense*, The Pannonian Plant Biotechnology Association (PPBA) Conference, Book of abstracts p. 41, 8-10 June, Ljubljana, Slovenia.
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Other conference participation:

1. **Lörincz-Besenyei, E.**, Mehdi, R., Sprink, T., Sonnewald, U. and Krenz, B. (2020) Developing a viral based genome editing tool for editing, Plant Biology Europe EPSO/FESPB 2020 (postponed to 2021 due COVID-19) Congress, Turin, Italy (**Awardee of the FESPB Support Grant**).
2. **Lörincz-Besenyei, E.**, Metje-Sprink, J., Sprink, T., Sonnewald, U. and Krenz, B., (2019) Inducing mutations in potato *via* genome editing in demand of climate change, COST Action 1st PlantEd Conference, Plant Genome Editing - State of the Art 5th – 7th November 2019 Novi Sad, Serbia-Abstract book pg 61.
3. **Lörincz-Besenyei, E.**, Mehdi, R., Sprink, T., Sonnewald, U. and Krenz, B., (2019) Tomato bushy stunt virus (TBSV) based ribonucleoproteins (RNPs) delivery in potato, 51. Jahrestreffen des arbeitskreises "Viruskrenkenheiten der Pflanzen", 25. bis 26. März 2019, Goettingen, pg.18.
4. **Lörincz-Besenyei, E.,** Sprink, T., Metje, J., Sonnewald, U. and Krenz, B., (2018) Potato improvement by genome editing, Nr. 200 (2018): 11th Young Scientists Meeting 2018, 14th-16th November in Braunschweig - Abstracts –pg.70.