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Decreasing of Monosaccharide Contents Resulted From Cold-Induced Sweetening in Tetraploid Potato (*Solanum tuberosum* L.) Using Genome Editing

Sally M Hassan[1]*, Mohamed A Rashed[2], Aiman H Atta[2], Haytham M Abd-Elhalim[3], Nouh E Ahmed[2], Emad A Metry[1]

1- Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza, Egypt
2- Genetics Dept, Fac of Agric, Ain Shams Univ, P.O. Box 68, Hadayek Shoubra 11241, Cairo, Egypt
3- School of Biotechnology, Badr University in Cairo, Badr City, 11829, Cairo, Egypt

*Corresponding author: sally_mohamed@ageri.sci.eg

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Abstract: Worldwide, potato (*Solanum tuberosum* L.) is one of the most essential agro-economically food crops. To ensure its presence throughout the year for food processors and to extend its shelf life, it should be stored in cold temperatures. Despite the benefits of storage at low temperatures, it causes undesirable phenomena; one of them is cold-induced sweetening, which reduces the quality and the commercial value of the potato tubers. In this study, the CRISPR technology ″Clustered Regularly Interspaced Short Palindromic Repeats″ has been used in potato cultivar *Desiree via Agrobacterium*-mediatied transformation to edit starch phosphorylase gene that is responsible for starch degradation in cold temperature storage, after regeneration steps. The regenerated plants were used in the screening of genome editing by Sanger sequencing and the ICE program. Screening results showed that *Desiree* 2 (DE2), *Desiree* 7 (DE7) and *Desiree* 27 (DE27) lines have mutations in sgRNA sequence that indicated a significant reduction in reduced sugar contents of tubers after cold storage for 90 and 120 days. The reduction mean values were 29.224, 35.078 and 35.862 in DE2, DE7 and DE27 respectively after 90 days and 83.212, 56.674 and 34.109 after 120 days without visible differences in plant morphology and tubers.

1 Introduction

Potato (*Solanum tuberosum* L.) is ranked the third-produced crop worldwide and produces more protein and dry matter than the most important cereal crops per hectare (Aksoy et al 2021). Nevertheless, the cultivation of potatoes is not continuous throughout the year due to the lack of favorable climatic conditions for plantation and thus farmers store tubers in cold temperatures. The advantages of cold storage are controlling sprouting and inhibiting the potato tuber's metabolism, except synthesis of sucrose which results from slow starch degradation with energy provided by the respiration of potato tubers, a phenomenon called cold-induced sweetening (Dite Hunjek 2021). The result of cold storage is the accumulation of both sucrose and its monosaccharides (glucose and fructose) resulting in the unbalance between starch degradation and glycolysis (Gupta et al 2019, Orzechowski et al 2021).

Genome editing is a big group of technologies and has several approaches that offer the ability to modify an organism's DNA without the insertion of foreign genes. These technologies have been developed to allow removing or adding genetic materials, or changing particular locations in the genome (Nadakuduti and Enciso-Rodríguez 2021). CRISPR and CRISPR-associated protein 9 is one of the genome editing techniques that generated a wide argument in the scientific community because of its more accurate, cheaper, faster and more efficient than other current genome editing methods (Mushtaq et al 2021).

The starch phosphorylase enzyme is one of the enzymes which are responsible for the diversion of starch to glucose during storage in low temperatures (Huan et al 2021), therefore we hypothesize that inhibiting the activity of this enzyme by CRISPR/Cas9 genome editing system would lead to the production of low-sugar potato tubers.

This study aims to inhibit the starch phosphorylase gene in potato cultivar *Desiree* using the CRISPR/Cas9 technique to decrease monosaccharides resulting from cold-induced sweetening during storage.

2 Material and Methods

2.1 Plant materials

Potato (*Solanum tuberosum* L.) (*Desiree* cultivar) is a tetraploid plant (2n=4x=48) acquired from Micro Propagation Technology Laboratory (MPT) stock in Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza-Egypt.

2.2 Cloning and expression vector construction

The used cloning vector was pChimera vector (2909 bp) which contains the single guide RNA (sgRNA) sequence that flanked by *Avr*II restriction enzyme sites and confers resistance to ampicillin. It was synthesized by Gene Art (Life Technologies Inc., Carlsbad, CA, USA) as presented in **Fig 1.**

pCAS9-TPC is a binary expression vector containing the Cas9 expression cassette for conventional cloning that is used for *Agrobacterium*-mediated transformation harbors and confers spectinomycin resistance in bacteria **Fig 2**.

Escherichia coli strain *DH10β* was obtained from Gene Silencing and Insect laboratory, AGERI. LBA 4404 *Agrobacterium* strain was obtained from Microbial Biotechnology and Molecular Biology laboratory, AGERI.

2.3 Bioinformatics analysis

The exon number 2 in the starch phosphorylase gene sequence was used as a template to design the sgRNA sequences using CRISPR-P 2.0 web-based tools. The sgRNA sequences were designed in Macrogen Lab., which used two complementary oligonucleotides, the forward (F1); 5' ATTGGCTGTTGCAAAGAATGCCTT 3' and the reverse (R1); 5' AAACAAGGCATTCTTTGCAACAGC 3' sequence.

The Sanger sequencing results for the edited plants were analyzed using the ICE program (Inference of CRISPR Edits). This program is a quantitative, quick and cheap assay program of editing outcomes that enables powerful analysis of CRISPR edits using Sanger data.

2.4 Cloning of sgRNA

2.4.1 Cloning in pChimera vector

Two µl of each oligonucleotide were mixed with 46µl of d.d H_2O and incubated for 5 min. at 95ºC, and then the mixture was kept at room temperature for 10 min. This mixture called annealed oligonucleotides (sgRNA) that was cloned through two steps, the first was the cloning of sgRNA into pChimera vector which was digested by *Bbs*I restriction enzyme, then ligated with annealed oligonucleotides using Bio Labs T4 DNA ligase kit. After that, the ligation reaction was transferred into a 150 µl vial containing *E. coli* competent cells for transformation according to Rahimzadeh et al (2016) that was spread on LB agar plates containing the appropriate antibiotic [Ampicillin (100 mg /l)] and then incubated for overnight at 37°C.

The transformed colonies (transformants) were detected by PCR analysis using F1 (forward primer of sgRNA) & M 13 (reverse primer of pChimera vector) **Table 1**. PCR reaction conditions were set at 94ºC for 3 min., followed by 30 cycles of denaturation at 94ºC for 30 sec.; then the annealing was set at 56ºC for 30 sec., followed by the extension at 72ºC for 40 sec. and finally, 7 min extension at 72ºC.

2.4.2 Cloning in pCAS9-TPC vector

The second step of cloning was performed into pCAS9-TPC vector; the miniprep of a positive colony and the vector were digested by *Avr*II restriction enzyme that was separated and purified using QIAquik®PCR purification kit (50) Qiagen (Cat. No. 28104). The resulting purified material was ligated by T4 DNA ligase and transfected into competent cells of *E.coli* using the heat shock method. After the transformation steps, the transformed competent cells were

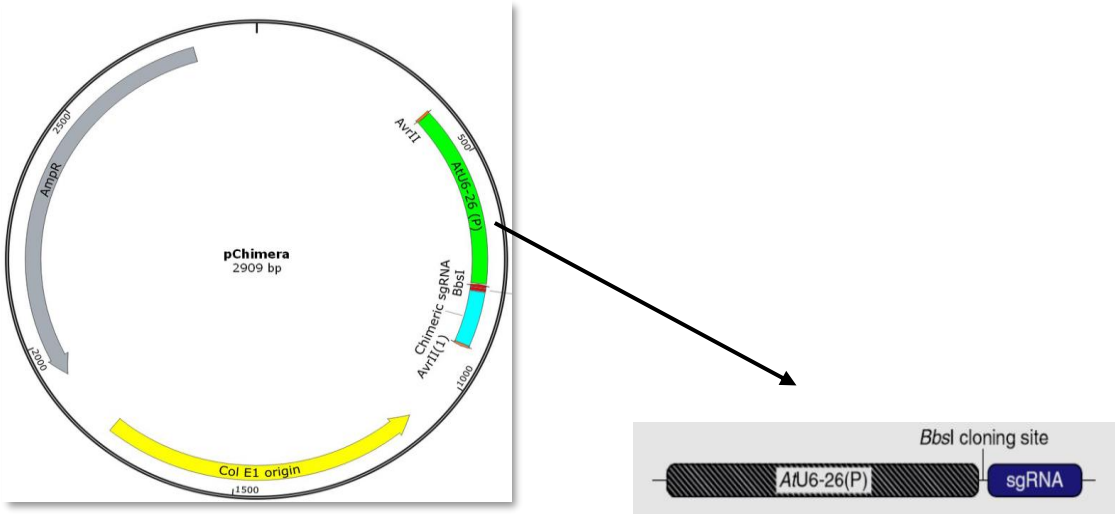


Fig 1. Schematic diagram of the pChimera cloning vector used for classical cloning. The BbsI recognition and cloning site that generate defined sticky ends. Two complementary oligonucleotides are cloned into the cutting site

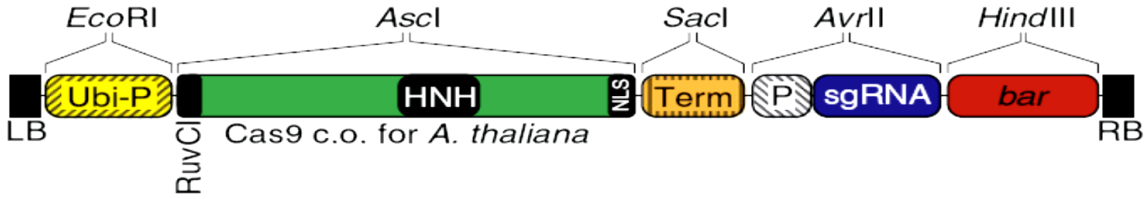


Fig 2. Schematic diagram of T-DNA constructs for nuclease of the pCAS9- TPC binary expression vector and restriction sites that used for *Agrobacterium*- mediated transformation

spread on plates supplemented with spectinomycin (50mg/l) and the transformed colonies were detected using SS42 forward & SS43 reverse primers **Table 1** after the incubation at 37°C overnight.

The positive colonies were transfected into *Agrobacterium* competent cells by heat shock steps, after that, 350µl of *Agrobacterium* cells was added to each LB agar plate supplemented with the spectinomycin (100 mg/l) and streptomycin (50 mg/l) then incubated for 2-3 days at 28° C. After that, the detection of transformed colonies was achieved by PCR analysis using SS42 forward and SS43 reverse primers.

2.5 *Agrobacterium*-mediated transformation of potato *(Solanum tuberosum* L.)

The Potato *Desiree* cultivar was chosen and micropropagated *in vitro* using the technique of nodal cutting described by Roca et al (1978). Every 3 to 4 weeks, nodal cuttings were routinely sub-cultured on a fresh MS salt medium (Murashige and Skoog 1962). Leaves of *Desiree* cultivar were transformed according to De Block (1988) by *Agrobacterium tumefaciens* containing pCAS9-TPC vector which carries a sgRNA sequence.

2.5.1 Callus induction and regeneration

The transformed leaves were incubated on the callus media containing MS media, a 5 ml/l of 2-4, D (1mg/ml) and a 1 ml/l of cefotaxime (200 mg/l) for 5 days at 25 ± 1 ºC in the dark. The leaves were transferred to a regeneration medium containing a 200 mg/l of cefotaxime, a 1 mg/l of IAA, a 1 mg/l of BA and a 10 mg/l of GA3 and incubated in the growth room at 20 ± 1 °C. After 72 days, shoots were transferred to a multiplication medium consisting of MS salts with 3 % sucrose and 2.6 g/l of phytagel and the pH was adjusted to 5.6 or 5.7. Numbers and percentages of the regenerated shoots were calculated and analyzed.

Table 1. Sequence of detection primers; M13 reverse: reverse primer of pChimera vector, SS42 forward & SS43 reverse: detection primers of pCAS9-TPC vector, sd1 forward & sd2 reverse: primers of partial sequence of starch phosphorylase gene containing sgRNA sequence with pam site and *bar* forward & reverse: detection primers (primers of selectable markers) of pCAS9-TPC vector

Primers	Nucleotide sequence	bp	Expected size
M13 reverse (R)	5'-CACAGGAAACAGCTATGAC- 3'	19	270 bp
SS42 forward (F)	5'-TCCCAGGATTAGAATGATTAGG- 3'	22	1000 bp
SS43 reverse (R)	5'-CGACTAAGGGTTTCTTATATGC- 3'	22	1000 bp
sd1 forward (F)	5'-CTATCATTCATTTTGAACCACATG- 3'	24	700 bp
sd2 reverse (R)	5'-TAATTTGTCAGCAAATATCAAACGG- 3'	25	700 bp
bar forward (F)	5'-TACATCGAGACAAGCACGGT- 3'	20	400 bp
bar reverse (R)	5'-ACGTCATGCCAGTTCCCGTG- 3'	20	400 bp

2.6 Genome editing confirmation of potato plants

2.6.1 Molecular analysis

DNA was extracted using QIAGEN DNAeasy Plant Mini kit (Cat. No. 69104) from potato *Desiree* cultivar (control and editing plants) that was used to detect genome editing by using 1.2% agarose gel with forward and reverse of *bar* gene primers. The conditions of PCR were started at 94ºC for 3 min., the 35 cycles of denaturation at 94ºC for 30 sec., the annealing at 56ºC for 30 sec. and extension at 72ºC for 40 sec., followed by 7 min extension again at 72ºC.

2.6.2 Bioinformatics analysis

Sanger nucleotide and amino acid sequence analysis was used in detecting the editing in the PCR mix of plants that used sd1forward and sd2 reverse primers. The automated DNA sequencing was performed by Macrogen Lab.; which focuses on providing research services in biotechnology research and development such as DNA sequencing and bioinformatics analysis. The substitution CRISPR edits were analyzed by Sanger sequencing and the percentages of indels (insertions or deletions) CRISPR edits were analyzed using Inference of CRISPR Edits software (ICE) which is an online tool.

2.6.3 Chemical analysis

Edited potato plant lines from *Desiree* cultivar (DE 2, DE 7 & DE 27) and non-editing control lines (DE) were planted in a greenhouse's pots with a (clay/ sand/peat-moss) growth medium to produce potato tubers using 3 to 5 plants (replicates) from each line.

The tubers were incubated at 20°C for one week in the dark and then transferred to a refrigerator at 4°C for 90 and 120 days to be used in the chemical analyses. The tubers were sliced and completely dried in an oven at 40°C for 3 days and then placed in tubes on the grinding machine.

Monosaccharides (reducing sugars) were extracted from stored tubers by using 80% ethyl alcohol and placed in a screw cap glass tube then heated to 70ºC in a hot plate or steam baths for 30 min. The phenol-sulfuric acid method was used to measure the monosaccharides according to Nielsen (2010). The overall reaction resulted in a yellow-orange color formation that was read spectrophotometrically at 490 nm.

3 Results And Discussion

3.1 Cloning of sgRNA

The first cloning step was for the annealed oligous with the pChimera vector that was digested by *Bbs*I, and measured on a nanodrop machine to ligate the vector and annealed oligous (1to 4 ratio) by T4 DNA ligase according to Hassan et al (2018).

The ligated product was transfected into DH10β competent cells of *E.coli*., while the opposite trend was reported by Taoutaou (2022) who transformed into GM2163 competent cells of *E.coli*. Screening for positive colonies (transformants) after cloning of sgRNAs in the pChimera vector was performed, carried out by colony PCR analysis using the primers of F1 forward and M13 reverse **Fig 3.** The transformants showed the expected fragment of 270 bp.

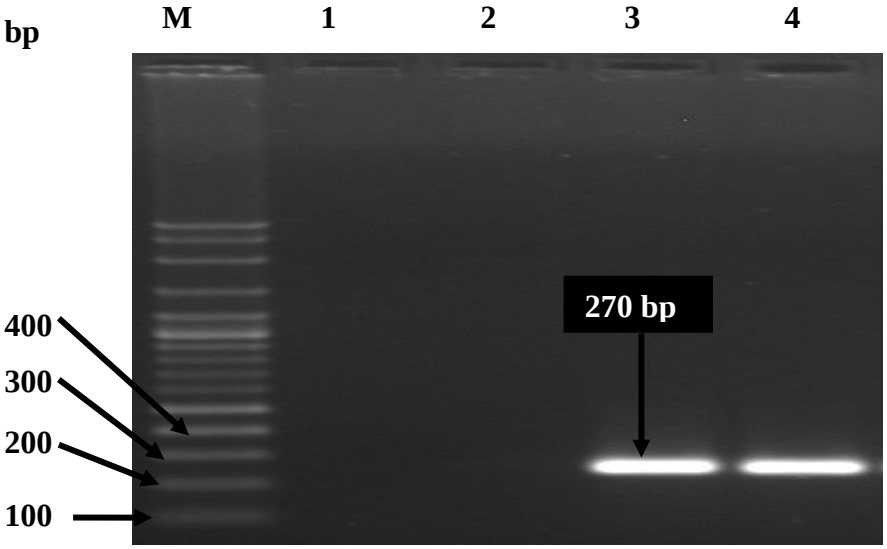


Fig 3. Screening of transformed (positive) colonies after transformation by *E.coli*. Lane1: negative control, Lane 2: negative colony, Lane 3 & Lane 4: positive colonies and M: 100 bp Ladder.

The second cloning step was in pCAS9-TPC vector, the transformation of positive colony number 2 into competent cells of *E.coli* (DH10*β*), the screening for transformed (positive) colonies used SS42 forward and SS43 reverse primers **Fig 4** in which the positive colonies showed a DNA fragment of 1kb size, the opposite trend was reported by Taoutaou (2022) who transformed into GM2163 competent cells of *E.coli*.

3.2 Transformation into *Agrobacterium tumefaciens*

pCAS9-TPC vector containing a sgRNA sequence was transformed into *Agrobacterium tumefaciens* strain (LBA4404) competent cells contrary to Gharghi et al (2023) who used *Agrobacterium tumefaciens* strain C58 for potato transformation. The transformed colonies were screened by using SS42 forward and SS43 reverse primers that showed the expected DNA fragment size of 1000 bp **Fig 5** which indicates that all colonies were transformants and carried the sgRNA sequence.

3.3 *Agrobacterium*-mediated transformation of potato *(Solanum tuberosum* L.)

The *Desiree* cultivar was used in genome editing according to Sevestre et al (2020). The *Desiree* cultivar was *in vitro* micropropagated using the nodal cutting technique. After 3-4 weeks; plantlet leaves were selected for transformation. The transformed leaves were incubated on the MS media for 1-3 days at 28ºC in the dark after transformation with LBA4404 *Agrobacterium* containing TpCAS9 vector.

3.3.1 Callus induction and regeneration

After one day of transformation, the transformed leaves of the *Desiree* cultivar were incubated on a callus medium (MS with 5mg/l of 2-4, D). In contrast Valkov et al (2021) used 2 mg/l 2, 4 -dichlorophenoxyacetic acid (2, 4-D) and 8 mg/l zeatin riboside (ZR).

Leaf explants derived from calli were placed on a regeneration medium for two weeks. The explants were transferred into fresh media. During the selection process successfully transformed calli continued to vigorously grow to produce shoot initiations, on the contrary Rashid et al (2021) used a medium containing appropriate concentrations of BAP and IAA without GA3. The steps of differentiation and elongation of the *Desiree* cultivar **Fig 6** and the callus induction results of the *Desiree* cultivar after 3 weeks recorded 100%. After 9-10 weeks of regeneration, these results decreased to 64%, in comparison with the results reported by Hassan et al (2018).

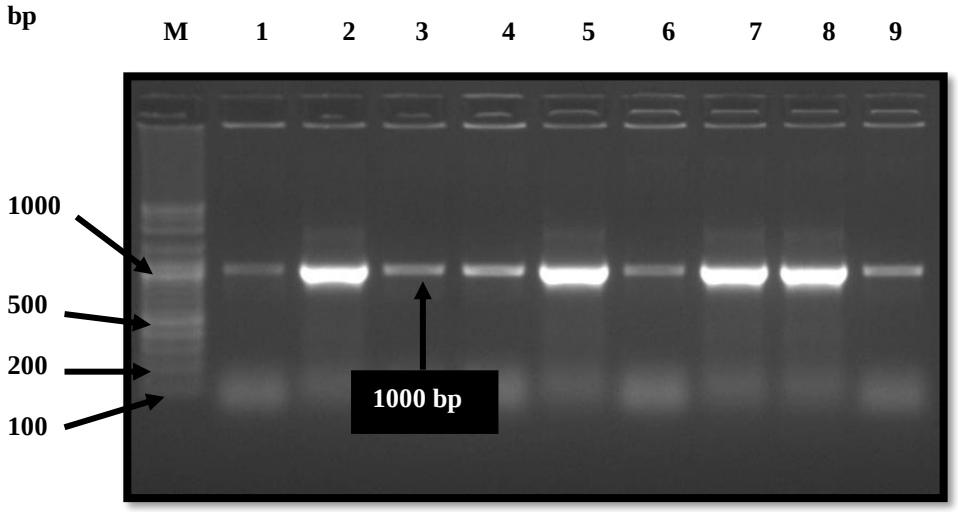


Fig 4. Screening of transformed (positive) colonies after transformation by *E.coli*. Lane1: positive control, Lane 2 to Lane 9: positive colonies and M: 100 bp Ladder.

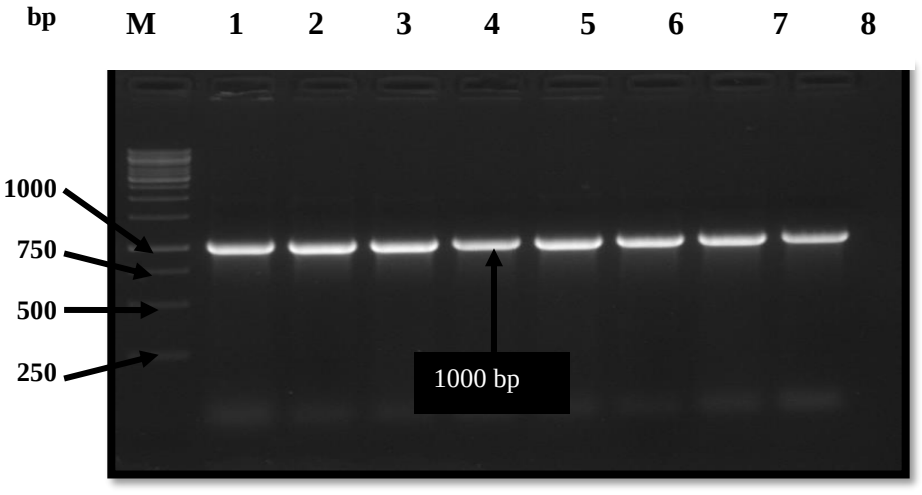


Fig 5. Screening of transformed colonies after transformation by *Agrobacterium.* Lane 1: positive control, Lane 2 to Lane 8: positive colonies and M: 1000 bp Ladder.

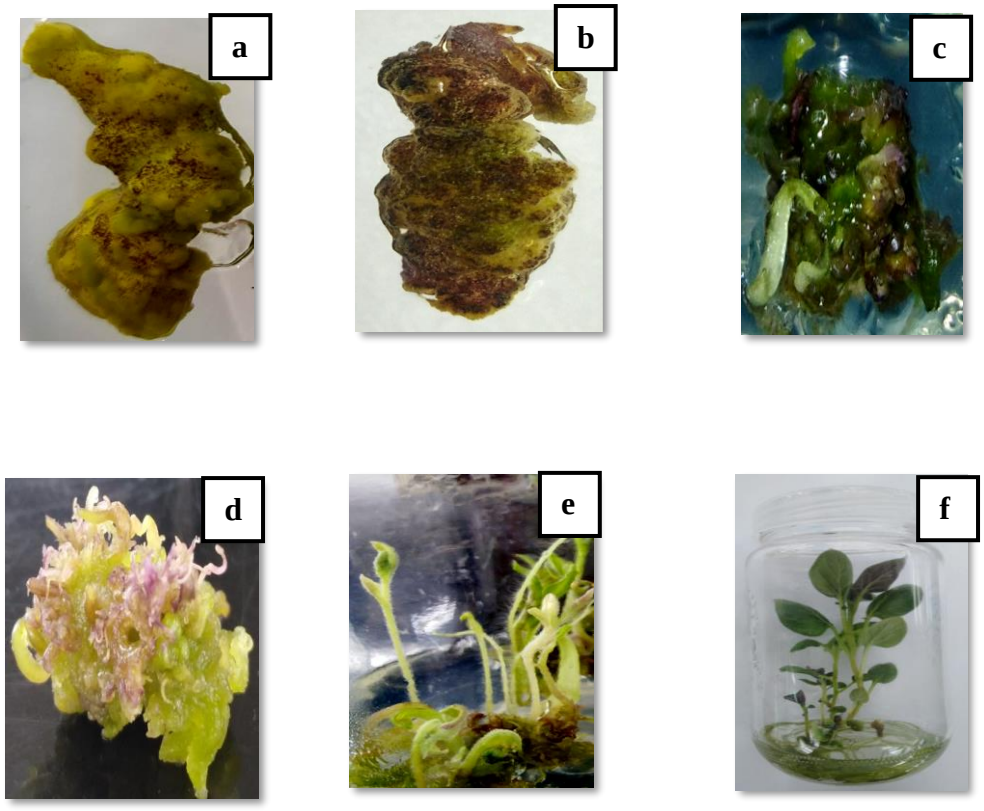


Fig 6. a, b, c, d, e and f steps of callus induction, regeneration and elongation of *Desiree* cultivar.

3.4 Genome editing confirmation

3.4.1 Molecular analysis

After DNA extraction from the transformed plants of *Desiree* cultivar, genome editing was confirmed using *bar* gene primers that showed a clear DNA fragment size of 400 bp in lane's numbers 4, 6, 11, 12, 25 and 27 clarify plants numbers 2,4, 11, 12, 24 and 26, respectively as shown in **Figs 7 and 8.**

3.4.2 Bioinformatics analysis

The results of Sanger sequencing showed that out of 29 plants, only three plants had indel and base pair substitution mutations by genome editing in the sgRNA region and the pam site of exon number 2 in starch phosphorylase gene that DE 2 and DE7 have deletion mutations and the DE 27 has substitution mutations as shown in **Table 2.**

The outputs files of ICE software that aid the user in checking the default indel, the quality of edits and the explanation of results as shown in **Fig 9** and the percentage of indels, knockout-Score, correction factor, R Squared and mean discord in edited plants as shown in **Table 3**, on the contrary, González et al (2020) used the High-Resolution Fragment Analysis (HRFA) to identify the edited lines and Yasmeen et al (2022) used TIDE analysis.

3.4.3 Chemical analysis

The first step of the chemical analysis was to produce the microtubers of genome editing lines and *Desiree* control in the greenhouse through the adaptation steps for four months, after that the microtubers were collected **Fig 10**.

The second step was quantifying the reducing monosaccharide contents in *Desiree* control and cold-stored genome-edited tubers by the phenol-sulfuric acid method after storage for 90 and 120 days at 4°C; the opposite trend was reported by Hameed et al (2018), who quantified the reducing sugars content in tubers after storage for 30, 120, and 180 days.

A standard curve, based on known concentrations of glucose, was prepared **Fig 11**. The obtained linear regression equation was used to calculate the concentrations of reducing sugars in the sample solutions.

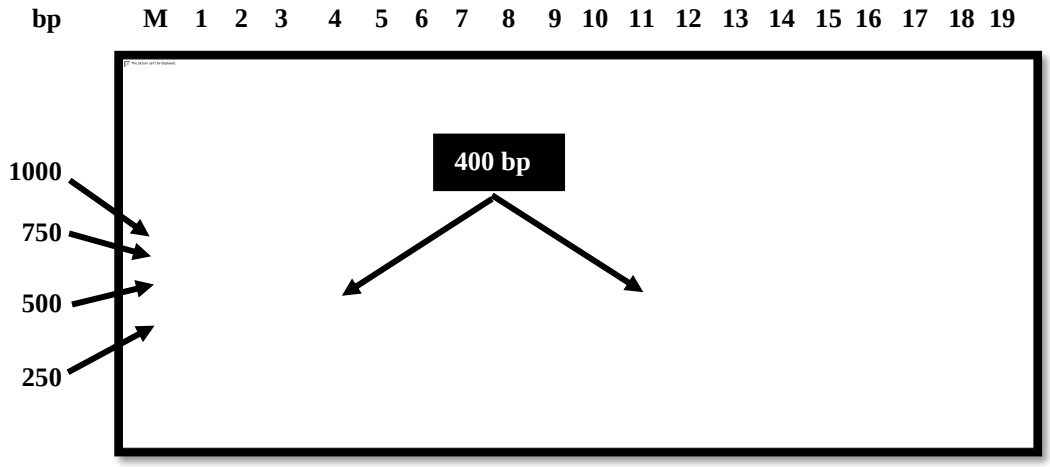


Fig 7. Confirmation of transformed plants by using forward and reverse *bar* gene primers in a 1.2% agarose gel and the expected size was at 400 bp. Lane1: negative control, Lane 2: *Desiree* control, Lane 4, 6, 11 and 12: containing *bar* gene and M: 1 kb Ladder

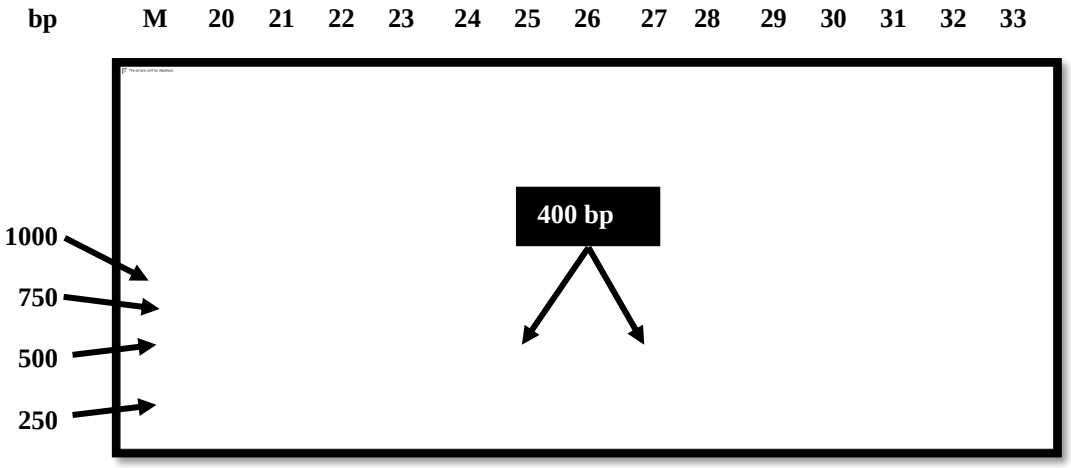
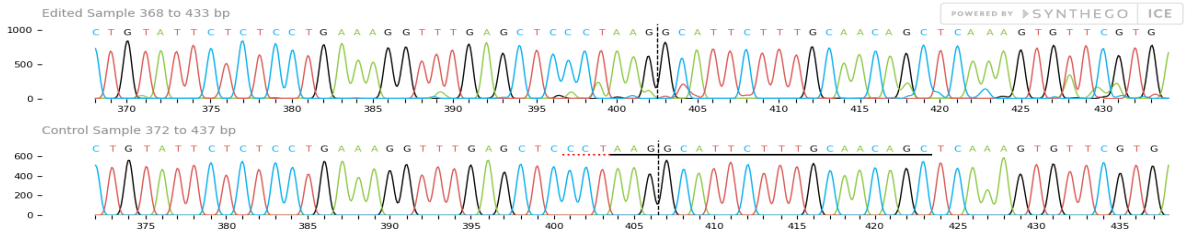


Fig 8. Remainder the confirmation of transformed plants by using forward and reverse *bar* gene primers and the expected size was at 400 bp. Lane 20: negative control, Lane 21: *Desiree* control, Lane 25 & 27: containing *bar* gene and M: 1 kb Ladder.

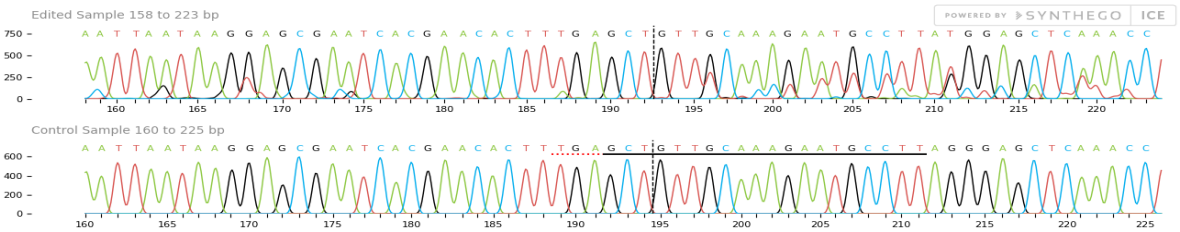
Table 2. Identification of edited lines and characterization of mutations by sequencing. The DE2 has deletion of four bases in allel and has substitutions in other alleles, the DE 7 has deletion of three bases in one allel and 23 bases in another allel and has substitution in another allel, and the DE 27 has substitution in three allels. The sequence of sgRNA is in bold letters, pam site is blue color and the edit action is in red color

No. of plant	Wild type (WT) and alleles	sgRNA		Indels	substitutions
DE 2	WT	5' CTT**TGAGCTGTTGCAAAGAATGCCTT**AGGGAGC	3'	0	0
	2-1	5' CTT**TGAGCT - - - - CAAAGAATGCCTT**AGGGAGC	3'	- 4	0
	2-2	5' CTT**TGAGCTGTTTCACCTATTTCTTT**ATGGAGC	3'	0	8
	2-3	5' CTT**TGAGCTGTTTCAAAGATTTCTTT**ATGGAGC	3'	0	5
	2-4	5' CTT**TGAGCTGTTTCAAAGAATGCCAT**ATGGAGC	3'	0	3
DE 7	WT	3' CTC**CCTAAGGCATTCTTTGCAACAGC**TCAAAGT	5'	0	0
	7-1	3' CTC**CC -** AAAGT	5'	- 23	0
	7-2	3' CTC**CCTAA - - - ATTCTTTGCAACAGC**TCAAAGT	5'	- 3	0
	7-3	3' CTC**CCTAAGGCATTCTTTGCAACAGC**TCAAAGT	5'	0	0
	7-4	3' CTC**CCAAAGGTATTCTTTGCAACAAC**TCAAAGT	5'	0	3
DE 27	WT	5' CTT**TGAGCTGTTGCAAAGAATGCCTT**AGGGAGC	3'	0	0
	27-1	5' CTT**TGAGCGGTTGCAAAGGGTGCCTT**AGGGAGC	3'	0	3
	27-2	5' CTT**TGAGCTGTTGCAAAGAATGTGTT**AGGGAGC	3'	0	2
	27-3	5' CTT**TGAGCTGTTGCAAAGAATGCCTA**AGGGAGC	3'	0	1
	27-4	5' CTT**TGAGCTGTTGCAAAGAATGCCTT**AGGGAGC	3'	0	0

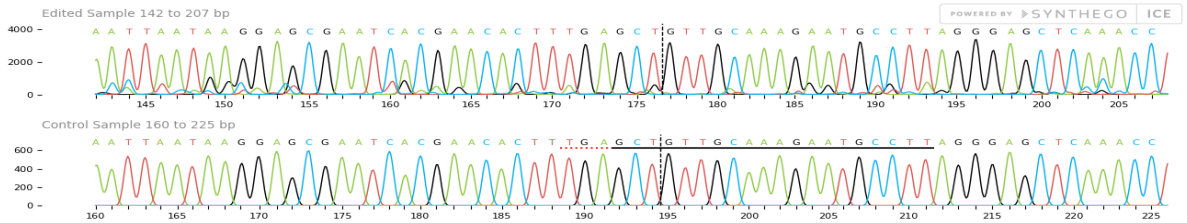
a-2)



a-7)



a-27)



b-2)

b-7)

b-27)

Fig 9. The ICE software outputs for a guide targeting the starchphosphorylase (sp) gene. a) Trace file clarifying the cut site from the control and the edited samples are generated for every analysis. The underline black is indicated to the guide sequence, and the red dots are indicated to the PAM sequence in the control sample. The expected cut site is denoted by vertical dotted lines b) The relative contributions show the indicated sequences present in edited plants and their relative proportions (in contrast to the Indel plot (Indel Distribution tab), The black vertical dotted lines are present the cut sites, and a "+" symbol on the far left is the wild-type sequence . a-2 and b-2): refer to plant no.2 (DE 2), a-7 and b -7): refer to plant no.7(DE 7) and a-27 and b -27): refer to plant no.27(DE 27).

Table 3. The percentage of indels, knockout-Score, correction factor, R Squared and mean discord in edited plants number 2, 7 and 27

	Plant no. 2 (DE 2)	**Plant no. 7 (DE 7)**	**Plant no. 27 (DE 27)**
ICE (Indel %)	1	6	0
KO-Score (Knockout-Score)	1	1	0
ICE d (correction factor)	14	23	13
R Squared (Model Fit)	0.93	0.94	0.96
Mean Discord Before	0.112	0.003	0.318
Mean Discord After	0.446	0.662	0.521

Fig 10. a, b, c, d and e steps of *Desiree* cultivar acclimatization in the greenhouse

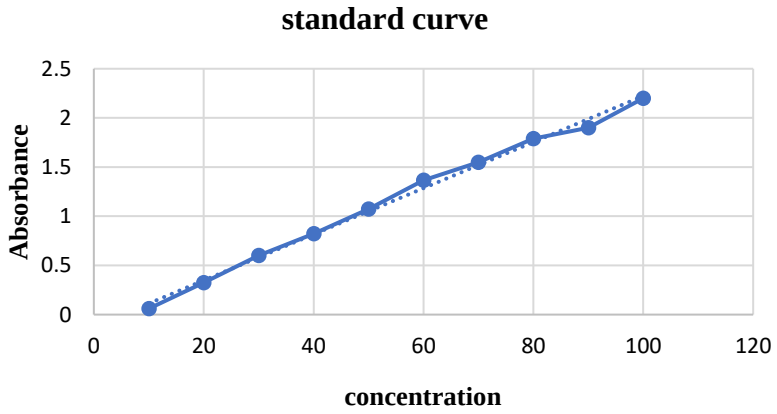


Fig 11. The standard curve was prepared with authentic glucose standard

The results showed a significant reduction in the level of reducing sugars in cold-stored tubers of the editing lines (DE 2, DE 7 and DE 27) compared with non-transformed (DE control), at $P \leq 0.001$ of reducing sugars that were recorded using the phenol-sulfuric acid method as shown in **Fig 12.**

Genome editing is a big group of technologies and has several approaches such as CRISPR/Cas9 technique (Clustered Regularly Interspaced Short Palindromic Repeats), TALENs (transcription activator-like effector nuclease) and ZFNs (zinc finger nucleases). CRISPR/Cas9, compared with other genome editing technologies, is simple, natural and cost-effective as well as has a high effect of mutagenesis and has been successfully used in some allopolyploid crops such as potato, wheat, soybean and cotton as reported by Janga et al (2017), Li et al (2017), Liang et al (2018), and Sánchez-León et al (2018).

In our study, the potato crop (*Desiree* cultivar) was used as a model for its ease of transformation and molecular studies of the potato. Previous studies Xu et al (2006), and Yu et al (2022), have shown that the potato tubers are stored at the temperature range of 4-8°C to reduce sprouting, water loss and pathogenesis, or stored in low temperatures (10°C) to reduce cold-induced sweetening, depending on the variety of the potato.

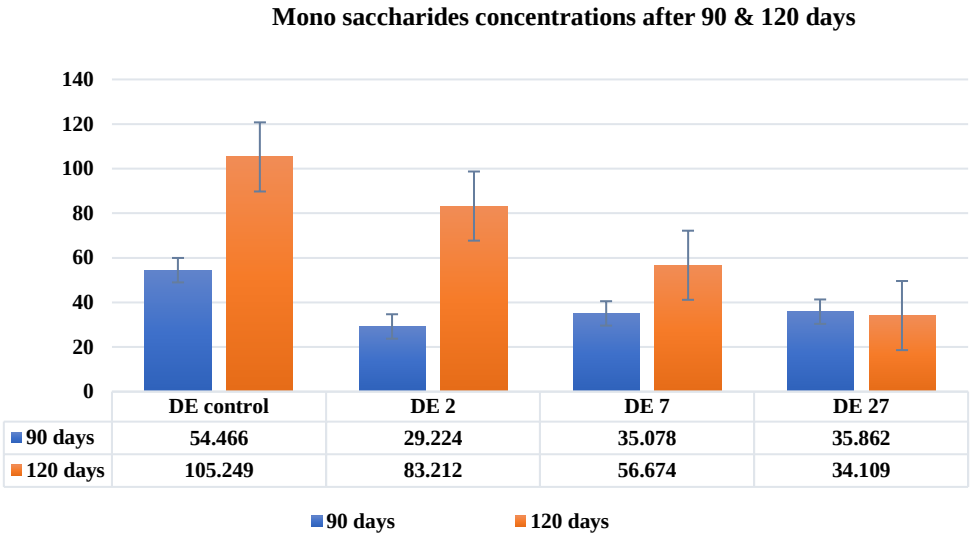


Fig 12. Bars represent the concentrations of monosaccharide contents in tubers after 90 and 120 days of storage and the mean ± S.D. in three replicates of independent samples relative to expression of the potato starch phosphorylase gene, $P \leq 0.001$.

In this study, we focused on the potato tuber storage at 4°C, which causes starch conversion to sugars, whether reducing sugars or sucrose. That conversion is known as the cold-induced sweetening phenomenon that affects potato tuber flavor.

The cold-induced sweetening phenomenon produces acrylamide formation which is considered ″Probable human carcinogen″ when these tubers are used in French fries and potato chips industries; thus, we focused on the cold-induced sweetening phenomenon in our study according to Shin et al (2002) and Yong et al (2018).

Several studies have targeted different genes in potatoes to induce mutations such as targeting the PPO2 gene (Hofvander et al 2021) and suppressing the *VInv* gene expression (Sowokinos et al 2001, Hameed et al 2020) to inhibit the cold sweetening process. However, in our study, the starch phosphorylase gene was used that catalyzes the starch and inorganic phosphate reaction to give glucose-1-phosphate by a reversible conversion that has an important role in the starch phosphorolytic degradation in cold low-temperature storage of tubers, and the synthesis of sucrose as documented by Sevestre et al (2020).

CRISPR/Cas9 system was used to induce mutations of the potato starch phosphorylase gene in a regenerating plant obtained after *Agrobacterium*-mediated gene editing *via* Cas9 and sgRNAs as reported by Ahmad et al (2022).

Furthermore, for the detection of mutation, (Fauser et al 2014) used high-resolution melting while Woo et al (2015), Malnoy et al (2016), and Hameed et al (2020) used the deep sequencing of targeted sites. The Sanger sequencing of PCR mix was used in our study for screening the genome editing in potato plants to identify the substitution mutations **Table 2**.

The presence of editing efficacy (indel mutations) was analyzed using the ICE algorithm program and the result was shown in **Fig 9** and **Table 3** while Yasmeen et al (2022) used TIDE analysis to screen the editing efficacy.

The phenol-sulfuric acid method was used for measuring the tuber's content of monosaccharides in edit lines; results clarified a significant correlation between cold-induced sweetening and duration of cold storage. In cold-stored edit tubers, the content of monosaccharides was significantly lower than that in the control tubers after 90 days of cold storage, as reported in the previous study by Hameed et al (2018).

After 120 days of cold storage, the levels of reducing sugars in edit lines were still lower than in control lines; the result was calculated and presented in **Fig 12**. These results are similar to that of Ly et al (2021) who reported that after cold storage, the cold-stored potato tubers had a significant reduction in the content of reducing sugars.

4 Conclusions

The CRISPR/Cas9 technique could be effectively applied to induce editing in plant genomes; the editing of starch phosphorylase gene by CRISPR/Cas9 system has an efficient effect on monosaccharide content in edited potato tuber lines that were stored at the low temperature of 4°C for 90 days and 120 days.

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