Chapter

# Prospects for Genome Editing of Potato

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## Abstract

Potato (*Solanum tuberosum* L.) is a staple food crop that could play a major role in improving food security in developing nations. The sustainable production of this crop faces many challenges like pests, diseases, abiotic stresses and post-harvest problems. Transgenic technology and gene silencing strategies offered a new hope of solution to the conventional time consuming breeding programmes. However the genetically modified crops are affected by regulatory approvals and safety concerns. In this aspect, gene editing techniques like ZFNs (zinc-finger nucleases), TALENs (transcription activator-like effector nucleases), and CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR associated Cas9), offer better choice for production of transgene and marker free disease resistant potatoes.

**Keywords:** Potato, ZFNs, TALENS, CRISPR/Cas9

## 1. Introduction

Potato (*Solanum tuberosum*) belonging to the Solanaceae family is the fourth most important staple food crop of the world consumed by more than a billion people [1]. The global total potato production exceeds 300 million metric tons [2]. Popularly called the 'poor man's friend', this crop can play a vital role to safe guard the food security and sustainability in the current scenario of surging population growth. The crop is a rich source of starch, vitamins especially C and B1 and minerals. It is also used for several industrial purposes such as for the production of starch and alcohol.

There is an urgency to increase the production and quality of potatoes to meet the demands of the rising population. However the development of new potato cultivars using traditional cross-breeding is complicated and slow due to tetrasomic inheritance and high heterozygosity of cultivated varieties [3]. Currently, research work using genome editing (GE) tools are being deployed for the precise improvement of desirable traits in crops. Genetically modified (GM) crop production faces many hurdles due to the complicated regulatory approval procedures whereas the technique of GE offers a better promise in crop improvement by making efficient and precise changes in the plant genome. This chapter describes the research advancements in potato using GE tools and the hurdles ahead due to the regulatory measures.

## 2. Constraints in potato production

### 2.1 Pests and diseases

Pests and diseases are major constraints to commercial production of potato. The major pests infesting potato include Colorado potato beetle (*Leptinotarsa decemlineata*), leafminer fly (*Liriomyza huidobrensis*), cyst nematodes (*Globodera pallida* and *G. rostochiensis*) and potato tuber moth (*Phthorimaea operculella*) during storage. The important diseases of potato include late blight (*Phytophthora infestans),* early blight (*Alternaria* ssp.), potato virus Y, potato leaf roll virus, bacterial wilt or brown rot' (*Ralstonia solanacearum*) and blackleg (*Pectobacterium carotovorum*) during storage.

### 2.2 Weeds

Weeds are a major problem in potato production and can reduce yields through direct competition for light, moisture, and nutrients, or by harbouring insects and diseases that attack potatoes. Weeds can have a detrimental impact on tuber yield when compared to potatoes grown in weed-free conditions [4, 5]. The weeds present at harvest can be detrimental to yield by increasing mechanical damage to the tubers and reducing harvesting efficiency by slowing the harvesting operation. Farmers mostly employ herbicides to enhance weed control.

### 2.3 Post-harvest shelf life

Postharvest management and storage of the potato is an important factor not only in preventing postharvest losses but also in maintaining its nutritional quality. This is because potato contains glycoalkaloids (GAs), a family of steroidal toxic secondary metabolites that occur in all parts of the potato. The levels of these toxins are significantly affected by postharvest handling stress factors with exposure to light, storage temperatures, and injuries/bruising being important stress factors. Storage is an important post-harvest activity in seed production. Storage under specific conditions is important to prevent excessive loss of weight as a result of drayage and to preserve germination quality. Prevention of diseases in storage is also important whether it be small farmer storage or commercial potato seed storage.

#### *2.3.1 Starch composition*

Potatoes are used for a variety of purposes, and not only as a vegetable for cooking at home. In fact, it is likely that less than 50 percent of potatoes grown worldwide are consumed fresh. The rest are processed into potato food products and food ingredients; fed to cattle, pigs, and chickens; processed into starch for industry; and re-used as seed tubers for growing the next season's potato crop. The commercial value of potato starch is governed by the proportion of its derivatives mainly amylose and amylopectin. There is much demand for amylose free potatoes in food and paper industries and more availability of potato cultivars with high amylopectin is warranted.

## 3. Genome editing and crop improvement

Crop improvement using conventional methods are often labour-intensive and time-consuming and the rarity and randomness of significant mutations to produce desirable traits hinder the development of new commercial varieties. Although

genetically modified crops were introduced since 1996, concerns have been raised regarding its safety and the regulatory measures adopted by different countries has hindered its popularity. However the use of genome-editing tools for crop improvement has gained much attention because of greater accuracy and efficiency compared to conventional breeding. Genome editing has revolutionised the field of agriculture. Genome editing methods utilise sequence – specific nucleases (SSNs). The potential of genome editing using various methods like Oligonucleotide Directed Mutagenesis (ODM), Zinc-Finger Nucleases (ZFNs), bacteria-derived Transcription Activator-Like Effector Nucleases (TALENs, based on protein–DNA interactions), Meganucleases (MNs), and Clustered Regularly Interspersed Short Palindromic Repeats (CRISPRs)/CRISPR-associated 9 (Cas9) endonuclease (an RNA-guided DNA endonuclease) system are being explored by many researchers because of availability of draft sequences of various crops in public databases. These methods make precise modifications in the target genome by DNA repair mechanism to produce transgene free genetically modified desired phenotypes. It is also possible to make epigenetic changes, where the DNA sequence remains unchanged but gene expression is altered because of chromatin changes that may be heritable. Targeted mutagenesis results in double-strand breaks (dsbs) at specific genomic locations [6] and this in turn induce either of the two native DNA repair mechanisms, namely:

*Non-homologous end-joining (NHEJ):* an imprecise repair mechanism that introduces variable length insertions or deletions at the breaking point, rendering the target gene non- functional.

*Homologous recombination (HR):* that inserts homologous DNA templates at the targeted point, allowing the precise insertion or deletion of nucleotides in a specific locus [7]. This technology has proven to be an efficient mechanism for genome editing, not only for model plant organisms, such as *Arabidopsis thaliana* and tobacco, but also for economically important crop plants, including soybean, corn and rice [8–11]. This method remains more complex as it requires the simultaneous delivery of a DNA repair template that carries the desired modification to be incorporated into the repaired locus [12]. However it has wider application in site specific gene insertion, stacking of genes at a specific genome position and genome alteration to a single base level [13].

### 3.1 Merits of genome editing

Genome editing as already mentioned is a precise breeding method that allows for targeted single gene modifications capable of altering gene expressions throughout the entire plant genome producing desirable outcomes. Random mutagenesis breeding method using radiations or chemicals on the other hand is undirected and alters thousands of genes [14].

Genome editing or 'precision genome engineering' method offers numerous applications like [15]:

- Improvement of crop yield in varying types of soil
- Production of plants more resistant to biotic and abiotic stress
- Development of plants with better root systems for nutrient uptake and the ability to source soil moisture
- Improvement of post - harvest storage
- Increase a plant's ability to sequester carbon. – research on modifying plants to increase their $CO_2$ fixation ability is underway in many laboratories [16]

Hence these novel biotechnological tools offers immense scope to meet the increasing demand of food supply by increasing the productivity of crops with the same level of resources and inputs.

## 3.2 Major genome editing tools

### *3.2.1 Zinc finger nucleases*

During the 1990s attempts were made by various researchers to improve the precision in genome editing with the discovery of zinc finger nucleases (ZFN). ZFNs are artificial restriction enzymes comprising of a specific zinc finger DNA-binding domain composed of 3-base pair site on DNA and a cleavage domain. The structure of ZFNs were engineered so that the DNA binding domain binds to specific DNA sequences in the genome and the cleavage domain cuts the DNA.at that specified location. The cleavage domain is a type II restriction enzyme (FokI endonuclease). Using this technique scientists can make a cut in the desired region thereby allowing to either delete the target sequence or insert a new DNA sequence via homologous recombination.

Multiple ZFNs can be combined to recognise longer sequences of nucleotides, increasing specificity and success rate of genome editing by 10 percent. The major drawbacks of ZFNs were:

- for each target a new ZFN had to be designed
- it was time consuming to engineer a successful ZFN
- poor targeting density and
- relatively high levels of off-target effects, leading to cytotoxicity

### *3.2.2 TALENs*

With the advent of time, transcription activator-like effector nucleases (TALENs) emerged as the more powerful tool in gene editing technology. TALENs are engineered from proteins found in nature and are similar to ZFNs in that they are composed of a non-specific cleavage domain from the type II restriction endonuclease FokI, fused to DNA-binding domain sequences. The engineering of these two domains resulted in stimulating NHEJ and HR leading to precise genome editing. The main difference is that each TALE domain recognise single nucleotides rather than relying on 3-base pair sites as in ZFNs. Hence, does not affect the binding specificity of neighbouring TALEs, making the engineering of TALENs much easier than ZFNs.

Forsyth and coworkers, demonstrated that the TALEN system could be used to successfully target T-DNA incorporation into a specific pre-chosen site in the potato genome that is transcriptionally active. Importantly, these investigators designed a vector that would not allow stable integration of the *TALEN* genes into the genome. Their data indicated that TALEN-induced integration of the gene of interest at specific sites, results in co-segregation and results in predictable expression level of the integrated gene [17].

Nicolia et al., employed site-directed mutagenesis in tetraploid potato through transient TALEN expression in protoplasts. The study highlighted that the site-directed mutagenesis technology could be used as a new breeding method in potato as well as for functional analysis of important genes to promote sustainable potato production [18].

TALENs are effective genome engineering technologies but their major limitation is that tailoring the DNA binding proteins to target a sequence of interest can be costly and time-consuming [19]. Furthermore, engineering TALENs to generate targeted DSBs requires two TALEN proteins capable of binding in a tail-to-tail orientation to facilitate the dimerization of FokI nuclease domain [20]. These and other, limitations were considerably reduced in the past few years due to the advent, development, and subsequent technological advancements of the CRISPR/Cas9 system [12].

*3.2.3 CRISPR*

CRISPR/Cas9 system is presently the widely used genome editing technology in wide range of species ranging from the smallest microbes to the largest plants and animals. Clustered regularly interspaced short palindromic repeats (CRISPRs) are a family of DNA repeats present in most Archaea and few bacterial species that act as molecular immunity systems against invading phages and nucleic acids. These distinctive loci consist of repetitive palindromic sequences (21–47 bp), separated by hypervariable spacer sequences that exhibit homology to exogenous viral and plasmid sequences, ranging between 21 and 72 bp. These arrays are often located adjacent to helper cas (CRISPR-associated) genes that encode polymerases, nucleases and helicases. When spacer sequences are transcribed, they generate small CRISPR-RNA (crRNA) fragments that hybridise with a small non-coding transactivating crRNA (tracrRNA). This double-stranded RNA molecule is used as a guide to target invading DNA sequences as a result of complementarity, and it directs the Cas9 endonuclease to these sequences for DNA degradation by double-strand cleavage at a site preceding the protospacer associated motif (PAM) [21].

The CRISPR/Cas9 genome editing technology has been successfully employed for the genetic editing of single or multiple gene targets in several plants, such as *A. thaliana,* tobacco, rice and sweet orange [10, 22–24] and for engineering of durable resistance, even at different levels of ploidy [25].

## 4. Genome editing in potato

Potato (*Solanum tuberosum)* is a heterozygous polyploid crop and this makes the introgression of valuable traits from wild varieties challenging and time-consuming task. Conventional breeding therefore failed when multiple traits or novel traits not present in germplasm need to be introduced for crop improvement. Availability of genome sequence data in public database and established genetic transformation and regeneration protocols has made potato a strong candidate for genome editing. These techniques can hence be utilised to improve the production and quality traits without impacting optimal allele combinations in current varieties [26–33]. The first successful demonstration of the use of TALENs in a tetraploid potato cultivar was by knocking out all four alleles of sterol side chain reductase 2 (StSSR2) [34] involved in anti-nutritional sterol glycoalkaloid (SGA) synthesis [35, 36]. In 2015, came an important breakthrough that both TALENs [18] and CRISPR/Cas9 [37] gene-editing systems could be used to efficiently modify the potato genome. In a tetraploid plant, instead of two copies (alleles) of any particular gene present in a diploid plant, there are four copies of the same gene. Advances in gene editing techniques have shown that for several polyploid plant species, rapid and efficient modification can be achieved for most, if not all, chromosomes in the multiple chromosome sets of polyploid plants [38]. In 2015, Wang and coworkers, conducted a study in potato and

demonstrated that the CRISPR/Cas9 system was highly efficient for targeted mutation of *StIAA2* gene encoding an Aux/IAA protein. They could obtain homozygous monoallelic and biallelic mutations in the first generation of transgenic plants [37].

## 4.1 Trait improvement in potato using genome editing

### *4.1.1 Disease resistance in potatoes*

Plant diseases cause a major constraint in potato production and incurs huge loss to the farming community. Researchers are yet to make a major breakthrough in producing potato resistant to viruses, bacteria and fungi using the gene-editing techniques. TALEN technology has already been successfully used for engineering bacterial blight resistant rice cultivars [10]. There has also been reports on the production of virus resistant plants using CRISPR/Cas9 method either by directly targeting and cleaving the viral genome, or by modifying the host plant genome to introduce viral immunity [39].

Late blight disease, caused by fungus *Phytophthora infestans*, is the major obstacle in increasing potato production [40]. Hence a major area of focus is the production of late blight resistant potato varieties by knocking out or removing disease susceptibility genes (*S*-genes) [41]. Currently the disease is controlled by fungicide spraying and breeding for disease resistance.

*R* genes (Resistance genes) encode R protein that degrades the toxin produced by the pathogen and initiates defence mechanism in plant. However there are chances of losing this resistance due to high rates of evolution of effector proteins by the pathogen. Genome editing method could be applied to produce late blight resistant potatoes by editing specific amino acids in *R*-genes essential for effector recognition. Another strategy for durable broad spectrum resistance is by loss of susceptibility [42]. Silencing of multiple susceptibility genes (*S*-genes) by RNAi resulted in late blight resistance in potato [43]. The drawback of RNAi is that it does not always result in a complete knockout. Genome-editing on the other hand by the introduction of both extracellular and intracellular receptors in potato cultivars can simultaneously knockout genes belonging to the S-locus, thus aid in attaining durable broad-spectrum resistance for late blight. Du and coworkers has reported the use of an extracellular receptor protein ELR (elicitin response) from the wild potato species, *S. microdontum,* in recognising an elicitin that is highly conserved in *Phytophthora* species offering a broad spectrum durable resistance to this pathogen [44].

The team led by Aman had reported the use of Cas13 for interference against Turnip Mosaic Virus (TuMV) expressing green fluorescent protein in *Nicotiana benthamiana* both in stable and transient systems. Various potato viruses like the *Potato virus X* (PVX), *Potato virus Y* (PVY) and *Potato leafroll virus* (PLRV) account for the low production of potato. So the above study raises the hope of employing CRISPR/Cas13a system in combating the pathogenic viruses [45].

### *4.1.2 Herbicide resistance in potatoes*

Butler et al., reported the creation of a single-stranded gemini virus-based DNA replicon (GVR) that carries *TALEN* genes targeting the potato *Acetolactate synthase1* (*ALS1*) gene and also a fragment of the *ALS1* gene that carries a mutation conferring tolerance to several classes of ALS-inhibiting herbicides. Transfection of potato cells with the gemini virus DNA replicon construct results in transient expression of TALEN genes. The double strand break created at the target site was repaired by Homologous recombination to recognise the ssDNA fragment of the *ALS1* gene carrying the desired mutation and integrate this new sequence in place of the wild-type

ALS1 sequence. The plants thus modified with GVRs did not have the presence of TALEN or gemini virus DNA sequence but held point mutations within ALS1 locus and exhibited significant tolerance to herbicide treatments [46].

#### *4.1.3 Improving post harvest shelf life in potato*

Potatoes are harvested only once annually and it therefore necessitates the cold storage of the tubers to extend its postharvest shelf life. This storage leads to the conversion of sucrose to reducing sugars (cold-induced sweetening (CIS)) that can, upon frying, lead to reactions with amino acids resulting in undesirable browning, creation of bitter tastes, and production of low amounts of toxic acrylamide. Clasen et al., targeted the vacuolar invertase (*Vinv*) genes of Ranger Russet potatoes for knockout using the TALEN gene-editing system to reduce CIS. Five out of 18 regenerated plants contained knockouts of all four *Vinv* alleles. Tubers from these plants contained no detectable reducing sugars, were light brown and after processing contained lower levels of acrylamide [30]. This *Vinv*-knockout potato was commercialised by Cellectis Plant Sciences (now Calyxt Inc.) [47].

#### *4.1.4 Modification of starch composition of potatoes*

Potato starch provides important nutrition for humans and animals besides its numerous industrial uses. The relative ratio of the two major starch types, amylose and amylopectin, determines the quality of potato starch. Hence controlling this balance has significant commercial applications. High amylopectin (amylose-free) starch has been an important common trait in staple crops due its commercial value in the food and manufacturing paper industries. In potato starchy tubers, the *GBSS* gene was successfully knocked-out to generate high-amylopectin potato using different gene editing tools.

Kusano et al. used the TALEN system to successfully disrupt copies of one key enzyme in the starch biosynthesis pathway, granule-bound starch synthase (*GBSS*) gene in potato protoplast cells [48].

In a study, Andersson et al. used transient expression of the CRISPR/Cas9 system to demonstrate complete knockout of all four GBSS alleles in PEG-treated potato protoplasts and in up to 2% of regenerated lines. The successful knockout of the *GBSS* genes completely resulted in only the amylopectin starch (amylose free) in regenerated potato microtubers [32]. In yet another study, Andersson et al. carried out a DNA-free genome editing method, using delivery of CRISPR-Cas9 ribonucleoproteins (RNP) to potato protoplasts, by targeting the gene encoding granule bound starch synthase (*GBSS*) [49].

Ma et al. used a non-viral, *Agrobacterium*-mediated infiltration method to express two TALENs with different molecular weights to target two endogenous genes -starch branching enzyme (SBE1)) and an acid invertase(INV2) into two vegetatively propagated potato cultivars, *Solanum tuberosum* Russet Burbank and Shepody. These TALENs, successfully agroinfiltrated and induced mutations at both targeted loci thus affecting the degree of branching potato cold sweetening. The agroinfiltration method was cheaper, less laborious and could save time as compared to the protoplast culture approach. The mutation was induced at the specific target site and this resulted in the production of improved plant varieties with less somaclonal variation [50]. Tuncel et al., demonstrated that Cas9-mediated mutagenesis of *SBE* genes has the potential to generate a range of new potato phenotypes with valuable starch properties without integration of foreign DNA into the genome [51].

Kusano et al. improved the gene editing system by fusing the translational enhancer dMac3 of the 5′ UTR of rice *OsMac3* mRNA to the 5′-end of Cas9 to

increase its level of expression. It was found that the Granule-bound starch synthase I (*GBSSI*) gene mutant frequency induced by CRISPR/Cas9 system was greatly increased and the mutant plants produced tubers with low amylose starch [52].

In 2019, Johansen et al., reported the improvement of CRISPR/Cas9 editing efficiency in the Granule-bound starch synthase gene at the protoplast level when Arabidopsis *U6* promoter was replaced by endogenous potato *U6* promoters. This team of researchers also used the Indel Amplicon Analysis (IDAA) technique for faster and direct assessment of insertions/deletions (indels) in plants with complex genomes like potato [53].

Sevestre et al. reported the successful usage of SNP physical map of *Solanum tuberosum* L. cv. Desiree revealing the position of diverse indels for designing a specific gRNA and knocked out an isoform of starch synthase *SS6* (gene), a key enzyme of the starch biosynthetic pathway [54].

Veillet et al. used the CRISPR-Cas9 base editing, precisely in the conserved catalytic KTGGL encoding locus of the StGBSSI enzyme using a cytidine base editor (CBE). This lead to the discrete variation in the amino acid sequence and loss-of-function allele producing plants with impaired amylose biosynthesis [55].

### *4.1.5 Production of SGA free potatoes*

Potato tubers accumulate steroidal glycoalkaloids (SGAs) α-solanine and α-chaconine that confer a bitter taste and exhibit toxicity against various organisms [56]. Commercial tuber production mandates a total glycoalkaloid content of less than 20 mg 100 $g^{-1}$ tuber fresh weight as per industry standards, but the SGA level should be higher in the aerial parts as it can act as an allelochemical to deter insect pests like Colarado potato beetle [57, 58]. Genome editing can be utilised to target specifically the tuber expressed or aerial parts expressed genes of the SGA biosynthetic pathway leading to the development of potato cultivars with low SGA levels in tubers while maintaining higher levels in the aerial parts.

Akiyama et al., from Japan reported the successful production of potato with reduced concentrations of the toxic steroidal glycoalkaloid (SGA) compounds, α- solanine and α-chaconine that accumulate in sprouts and green tubers by genome editing. The team applied CRISPR-Cas9 system to knockout the potato *CYP88B1* gene involved in a later step of the SGA biosynthetic pathway. The *CYP88B1*-knockout potatoes showed no accumulation of SGAs. Furthermore, the corresponding amounts of steroidal saponins, important compounds in the pharmaceutical industry, accumulated in the knockout potatoes as a result of the decrease in SGA synthesis [59].

Nakayasu et al., and Yasumoto et al., used TALEN and CRISPR/Cas9 to knockout the *SSR2* gene encoding for sterol side chain reductase 2 and the *St16DOX* gene encoding for the steroid 16α-hydroxylase in the SGA biosynthetic pathway. This prevented SGA accumulation in potato tuber and hairy roots, respectively [60, 61].

### *4.1.6 Reduction of enzymatic browning in potato tubers*

Polyphenol oxidase (PPO) catalyses the conversion of phenols to quinones resulting in browning and reducing the devaluation of the processed products from the tubers. TALEN methods was employed to knock out one of the PPO genes in potato tubers resulting in decreased browning. This technique was commercialised using different delivery techniques (PEG-mediated transfection or *Agrobacterium*-mediated transformation) by two companies (Calyxt Inc., and Simplot Plant Sciences).

Gonzalez et al., produced potatoes with reduced browning by specific editing of the polyphenol oxidase gene (*StPPO2*) in the tetraploid cultivar Desiree. CRISPR/Cas9 system using RNPs as a delivery system was employed to induce mutations in

the *StPPO2* gene resulting in the production of lines with a reduction of up to 69% in tuber PPO activity and a reduction of 73% in enzymatic browning, compared to the control [62].

Khromov et al. compared *in vitro* activities of various sgRNAs designed for different regions of *phytoene desaturase* (*PDS*) from the carotenoid biosynthesis pathway and a *coilin* gene involved in plant resistance. The visual phenotype of PDS knockout makes it convenient for detection and analysis of potato genome editing due to the depigmentation in the absence of PDS. Knockout of *coilin* gene is highly desirable as deterioration of coilin is mainly involved in pathogen resistance and improving tolerance to biotic and abiotic stresses. The study revealed that the first six nucleotides located in the DNA substrate proximal to the 3'PAM site directly binded with Cas9 but did not affect the activity of Cas9-sgRNA complex. The researchers drew a conclusion that the unpaired nucleotides of target DNA with sgRNA can both stimulate or repress the activity of Cas9-sgRNA complex *in vitro* depending on the position of the mismatch [63] (**Table 1**).

### 4.2 Challenges in genome editing of potato

Potato is a clonally propagated highly heterozygous polyploid crop and hence complicates the use of gene editing techniques- difficulty in target designing for genome editing, obtaining homozygous mutants with all target genes mutated.

This mandates the need for screening large number of transformants to identify and propagate multiallelic mutagenic lines. Another challenge is that not all cultivars of potato are amenable to transformation and others need to be tested for transformation and regeneration in tissue culture. Protoplast transformation and regeneration of plants from leaf protoplasts also can lead to somaclonal variation, which may have negative impact(s) on plant development [67].

Attempts are being made by breeders to develop diploid potato lines inorder to understand complex agronomic traits. A major obstacle in potato breeding was the development of inbred lines due to self-incompatibility that hinders the fixing of gene edits and selection of progeny by segregating out the inserted foreign gene. Ye et al. developed self-compatible diploid potatoes by knocking out the self-incompatibility gene, Stylar ribonuclease gene (*S-RNase*) using the CRISPR-Cas9 system. This strategy opens new avenues for production of diploid inbred and self- compatible potato germplasm and pave way for studying other self-incompatible crops [66].

However many diploid, self- compatible potato germplasm were found to be recalcitrant to conventional *Agrobacterium tumefaciens* -mediated transformation [68]. Butler et al. demonstrated the utility of *A. rhizogenes* strains for rapidly generating stable mutations within hairy root clones in potato genotypes recalcitrant to *A. tumefaciens* and regenerating fertile lines capable of fixing targeted mutations, segregating out T-DNA insertions and production of additional mutants when needed. There is however a limitation to analysis of hairy root clones. CRISPR/Cas9 technology was successfully employed for targeting the potato *phytoene desaturase* (*StPDS*) gene, expressed in hairy root clones and regenerated. Targeted mutation was expressed in 64–98% of the transformed hairy root clones and this broadens the potato genotypes amenable to *Agrobacterium*-mediated transformation while reducing chimerism in primary events and accelerating the generation of edited materials [69].

Another area of concern is the occurrence of off-target mutations in non-target genes of potato during the process of GE. This results in undesired changes in plants and makes the process of mutational analysis studies more complicated. Attempts have been made to reduce or even eliminate such off-targeting by good design and test of sgRNA activity [70] and use of synthetic proofreading Cas9 variants [71].

| Target Gene | Function of Target gene | Gene editing method | Gene delivery method | Trait improved | Reference |
|---|---|---|---|---|---|
| Sterol side chain reductase2 (*StSSR2*) | Steroidal glycoalkaloids reduction in tubers | TALENS | Agrobacterium | Identify key enzyme in the biosynthesis of cholesterol and related steroidal glycoalkaloids | [34] |
| Acetolactase synthase 1(StALS1) | Herbicide resistance | TALENS | Protoplasts | Transient expression of TALENS in potato protoplasts for targeted mutagenesis and regeneration | [18] |
| Acetolactase synthase 1(StALS1) | Herbicide resistance | CRISPR/ Cas9 | Agrobacterium Gemini Virus Replicon (GVR) | Targeted mutagenesis and germline inheritance | [64] |
| Auxin/Indole 3 Acetic Acid (IAA) protein (StIAA2) | Petiole hyponasty and shoot morphogenesis | CRISPR/ Cas9 | Agrobacterium | Targeted mutagenesis in the first generation of transgenic plants | [37] |
| Vacuolar invertase (StVInv) | Cold induced sweetening , acrylamide content in tubers | TALENS | Protoplasts | Tuber improvement for cold storage | [30] |
| Acetolactase synthase 1(StALS1) | Herbicide resistance | TALENS | Agrobacterium | For targeted T-DNA integration | [17] |
| Acetolactase synthase 1(StALS1) | Herbicide resistance | CRISPR/ Cas9 TALENS | Agrobacterium Gemini Virus Replicon (GVR) | Gene targeting via homologous recombination using donor template | [46] |
| Granule bound starch synthase (StGBSS) | Tuber starch quality | TALENS | Agrobacterium | Development of a Gateway system for rapid assembly of TALENS in a binary vector | [48] |
| 1,4 alpha –glucan branching enzyme gene (SBE1), Vacuolar invertase (StVInv) | Degree of starch branching, cold induced sweetening | TALENS | Agroinfiltration | Effective delivery of TALENS and induction of mutation | [50] |
| Granule Bound starch synthase (StGBSS) | Tuber starch quality | CRISPR/ Cas9 | Protoplasts | Targeted mutagenesis and regeneration resulting in tubers with high amylopectin starch | [32] |
| Transcription factor gene (StMYB44) | Phosphate transport via roots | CRISPR/ Cas9 | *Agrobacterium* | Understand the molecular basis of phosphate stress responses | [65] |
| *CYP88B1* | Involved in later step of steroidal glycoalkaloid (SGA) pathway | CRISPR/ Cas9 | *Agrobacterium* | Absence of steroidal glycoalkaloids | [59] |

| Target Gene | Function of Target gene | Gene editing method | Gene delivery method | Trait improved | Reference |
|---|---|---|---|---|---|
| Granule Bound starch synthase (StGBSS) | Tuber starch quality | CRISPR/ Cas9 | Protoplasts Ribonucleoproteins (RNPs) | Regeneration of mutant lines without amylose | [49] |
| Steroid 16α hydroxylase (16DOX) | Encodes a steroid 16α-hydroxylase in SGA biosynthesis | CRISPR/ Cas9 | *Agrobacterium rhizogenes* | Absence of steroidal glycoalkaloids-α solanine in hairy roots of potato | [60] |
| Stylar ribonuclease (S-Rnase) | Self incompatibility | CRISPR/ Cas9 | *Agrobacterium* | Self compatibility in diploid potato lines | [66] |
| Granule bound starch synthase (StGBSS) | Tuber starch quality | CRISPR/ Cas9 | *Agrobacterium* | Reduced amylose starch in tubers | [52] |
| Phytoene desaturase (PDS) and coilin gene | Carotenoid biosynthetic pathway and biotic stress resistance | CRISPR/ Cas9 | | Loss of colour and enhances resistance to biortic sress | [63] |
| Sterol side chain reductase (SSR2) | Encodes key enzyme in steroidal glycoalkaloid (SGA)synthesis | TALEN | *Agrobacterium* | Reduced steroidal glycoalkaloids | [61] |
| Starch branching enzymes (SBE1 and SBE2) | Introduction of α 1,6 linkages in starch | CRISPR/ Cas9 | *Agrobacterium*/ Protoplasts | To generate tubers with a wide range of desirable starch content | [51] |
| Granule Bound starch synthase (StGBSS) | Tuber starch quality | CRISPR/ Cas9 | Protoplasts with StU6 endogenoue prpmoter | Reduced amylose starch in tubers than the previous studies with foreign promoter | [53] |
| Granule Bound starch synthase (StGBSS) | Tuber starch quality | CRISPR/ Cas9 | Agrobacterium | plants with impaired amylose biosynthesis; Base editing in conserved catalytic KTGGL encoding locus of the StGBSSI enzyme | [55] |
| Polyphenol oxidase (PPO2) | Conversion of phenolic substrates to quinones leading to browning | CRISPR/ Cas9 | Protoplasts Ribonucleoproteins (RNPs) | Reduced browning | [62] |

**Table 1.**
*Crop improvement in potato by Gene editing techniques*

A major area of focus is the generation of transgene free potato. Inorder to be accepted by the public and regulatory bodies, there should not be any trace of the exogenous DNA in the GE crops. Segregation of genetic lines is used in generation transition from T0 to T2, so that stably inherited transgene-free plants can be obtained in T2 mutant lines [72]. However, this strategy cannot easily be adopted in tetraploid potato with high allelic polymorphism. RNP delivery into protoplasts is now emerging as an excellent alternative system that avoids DNA intermediates [73].

## 5. Regulations on genome edited crops

The cultivation and commercialization of genetically modified crops did not attain the expected growth as it received a setback due to the strict regulations imposed by various countries. With the advent of the gene editing techniques, attempts were made to produce genome modified plants without exogenous DNA so that they do not come under the purview of the regulations.

Regulatory approaches for genome edited products is still in its infancy and different countries have issued their own legal interpretations. Different countries have adopted regulation on genome edited crops based on two types of regulatory frameworks: process-based and product based. In the case of process-based regulation, regulation is typically triggered if nucleic acids are introduced into crops or recombinant DNA technologies are deployed in the development of a crop. The European Union (EU), Argentina, Brazil and several other countries have a process-based regulatory framework [15]. EU declared that the genome edited plants can alter the natural genetic material of the plant producing adverse environmental issues and hence should be treated as transgenic plants. This stringent approach can hinder research in the development and also impact the trade of gene edited crops.

In the case of a product based regulatory framework the focus is placed on the risk inherent in the final product. The United States which has a product-based regulatory framework has no regulation for genome edited plants if no genetic elements from pathogenic species or pesticidal traits are introduced [74]. Multiple level checks are followed like FDA weighs on health benefits and the EPA weighs on the environmental impact of the edited crops. Null segregants – progeny of the transgenic, edited parent that still retain the germline edit but lack the integrated foreign DNA sequence – are exempted from regulation. Clonally propagated plants like potato normally does not produce null segregants. Japan also adopted a regulatory policy similar to the United States stating that the gene-edited plants in Japan should not be regulated (The Scientist news). Although the products of rDNA technology will still be regulated, it was stated that the genome editing technologies poses no increase in risk and therefore do not require additional regulatory oversight. No regulations were imposed by USDA on anti-browning mushrooms developed by targeting PPO using CRISPR/Cas9, indicative of the acceptance of traits created by gene editing [75].

The world's first regulation for GE crops was reported by Argentina [76]. Later on, Brazil and Chile adopted the same policies. Currently, many countries do not have a clear regulatory framework for GE crops. However, several countries like Kenya, Nigeria, and India are in the process of developing the regulatory guidelines for the application of genome editing [77].

### 5.1 Impact of the regulations

The commercialization of genome edited crop poses a challenge to the public sector breeders who lack funding, if they are treated equivalent to GM crops. The uncertainty in regulations will also have logistical challenges for international

commodity trade. The application of genome editing can reap its benefit and ensure agricultural sustainability depending mainly on the regulatory measures adopted by each country. The potential of genome editing can be exploited fully only if it is not treated on par with genetically modified plants and not subjected to the same regulatory measures.

Another constraint in the deployment of gene editing technology is the lack of a clear implementation and effective management strategy for the sustainable development of crops produced using this tool. Do we have to adopt the practice of crop monoculture inorder to harbour durable resistance is a question under debate? From the sociological point of view also, the public acceptance of food crops engineered using genome editing technology also needs to be considered.

## 6. Conclusion

Genome editing could play a major role in the modification of starch content, decrease antinutrient and toxic substances and enhance the nutritive value of potatoes. This technology with high efficiency and precision raises the scope of improving other desirable plant traits. The research advancements in this field can be accelerated by the production of transgene free GE potatoes and the commercialization of the technology can be promoted only by assuring the public of its safety. Despite the challenges faced in the commercialization of GE crops and its products, intense research is being carried out in different countries. Attempts to exclude GE crops from the GMO regulations raises hope in the advancement of the editing related technology. The availability of whole genome sequence of potato, transformation and regeneration protocols of potato, and novel gene editing tools instills hope of producing elite transgene free potato plants with desirable traits in short span of time.

## Conflict of interest

The authors declare no conflict of interest.

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