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Trends in
Genetics

Forum

Potato trait development going fast-forward with genome editing

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Implementations and improvements of genome editing techniques used in plant science have increased exponentially. For some crops, such as potato, the use of transcription activator-like effector nucleases (TALEN) and clustered regularly interspaced short palindromic repeats (CRISPR) has moved to the next step of trait development and field trials, and should soon be applied to commercial cultivation.

Potato, an important food and starch crop

Global potato production has steadily increased over the past 30 years and it is now one of the top-three staple food crops. Potato is also one of a handful of crops grown for the production of starch, a renewable bulk product used in various food and non-food applications. Cultivated potato is tetraploid, highly heterozygous, and vegetatively propagated. Therefore, the development of new cultivars using traditional breeding methods is a long-term effort. Extending the breeding toolbox with technologies that can introduce genetic diversity to potato genotypes without interrupting the overall genetic context is thus particularly beneficial for potato. Here, **genome editing** (see Glossary), especially TALEN- and more recently CRISPR-based methods, have shown the potential to become the next established breeding tools (Box 1). This is evident in the fact that most recent studies in potato include the development of novel commercial traits, of which we highlight a selection of in this forum.

Potato with increased tolerance to biotic stress and herbicides

Potato is prone to many diseases, and the use of fungicides is being questioned. At the same time, a number of pathogens are rapidly evolving; therefore, different breeding strategies, particularly using genome editing techniques (Box 2), could be a way to keep pace with this evolution from the plant side. A general approach for increased resistance is the inactivation of so-called susceptibility genes. This has recently been tested by screening seven different potato genes by ***Agrobacterium*-mediated transformation** of CRISPR-**CRISPR associated protein 9 (Cas9)** and resulted in potato plants with increased, but not full, late blight resistance [1]. The inactivation of genes that were shown to give increased resistance without apparent growth defects were *DOWNY MILDEW RESISTANCE 6* (*DMR6-1*) and a bHLH transcription factor (*CHL1*). Inactivation of susceptibility genes can provide broad resistance against several diseases, has the potential to be a fairly sustainable technique, and can be combined with other sources of resistance. Another approach aimed to restore truncated proteins in a defense pathway by **single nucleotide polymorphism** replacement in a caffeoyl-CoA *O*-methyltransferase gene using CRISPR-Cas9. This technique has been described to offer partial resistance to late blight [2]. However, documented field data from all of these potato studies are currently lacking.

Herbicide tolerance was targeted early in the implementation of TALEN and CRISPR in potato, where point mutations in an *ACETOLACTATE SYNTHASE* (*ALS*) gene were induced. In addition to being a commercially interesting trait, it is advantageously used during methodological developments, since the trait and induced desired mutations can be conveniently

Glossary

***Agrobacterium*-mediated transformation:** a method for inserting new genetic material in the genome. The new genetic material is inserted at a random site in the genome.
Base editing (BE): a method for the conversion of a base (e.g., C→T, A→G) to induce point mutation at a specific site in DNA. The system is based on CRISPR in combination with a single-strand-cutting nuclease and a deaminase.
Cisgenic: an organism that has genetic elements from its own or crossable species transferred to its genome.
Clustered regularly interspaced short palindromic repeats (CRISPR): an immune system found in bacteria that has been adapted to a genome editing technique. A CRISPR-derived RNA can be engineered to find and bind to a specific site in an organism's genome. This guide RNA can, together with a nuclease (e.g., Cas), induce mutations at a specific site in the genome.
CRISPR associated protein 9 (Cas9): a nuclease cutting double-stranded DNA, generating DSBs.
Double-strand break (DSB): a break in both strands of DNA. A DSB is repaired by the cell's own DNA repair mechanism (i.e., NHEJ or HR).
Genome editing: targeted changes made in an organism's genome by various techniques (e.g., TALEN- and CRISPR-based methods).
Glycemic index: gives a value of how quickly the intake of a food causes an increase in blood glucose levels.
Homologous recombination (HR): transfer of genetic material between two homologous strands. In genome editing, this process can be used by applying a repair guide with homologous arms to the target site, also known as homology-directed repair.
Nonhomologous end joining (NHEJ): an error-prone DNA repair pathway after DSBs that can lead to mutations in the genome.
Prime editing (PE): a CRISPR-based method using a single-strand-cutting nuclease together with a reverse transcriptase and a PE RNA (pegRNA), with dual functions as both target and repair guide for the genetic edit; can be used to induce designed edits in the genome.
Ribonucleoprotein (RNP): a complex of RNA and RNA-binding protein; for genome editing, can be used by assembling a CRISPR-Cas complex *in vitro* following transfection to cells. Hence, this DNA-free system ensures that no foreign DNA can be inserted in the genome, avoiding transgenesis.
Single nucleotide polymorphism: a single-base variation in a gene or an allele in a genome.
Transcription activator-like effector nucleases (TALEN): a protein-based genome editing method using a designed protein TAL effector DNA-binding domain together with a nuclease to target and induce mutations at specific sites in a genome.
Transgenic: an organism that has foreign genetic elements stably inserted in the genome.
Transient expression: temporary expression of genes in a cell. The genes can, for example, encode TALEN and CRISPR tools.

Box 1. Genome editing for potato breeding: potentials and challenges

To date, the majority of traits added to potato using TALEN or CRISPR are based on induced **double-strand breaks (DSBs)** and DSB repair by error-prone **nonhomologous end joining (NHEJ)**. Both the stable introduction of genome editing-tools in the genome, using *Agrobacterium*-mediated transformation, and vector and RNP-based transient application of the same tools result in a very high mutagenesis efficiency when applied to potato. Although transient application of genome editing has benefits such as low risk of obtaining chimeras and reduced risk of induced off-targets as well as yielding transgene-free events, stable transformation is today the predominant delivery method in research studies, plausibly due to the cumbersome process of isolation and regeneration from protoplasts. Also, regeneration from protoplasts can sometimes lead to somaclonal variation with effects on plant development, which needs to be taken into consideration during both research and breeding projects. A benefit of using genome editing compared with more traditional gene inhibition technologies is that full knockout of an enzyme can be ensured. However, complete loss of an enzymatic activity can occasionally yield unwanted secondary effects. Hence, partial enzymatic inhibition or a translated inactive enzyme might be beneficial for some trait developments. This can be obtained by combining designed sgRNA targeting with the selection of desired mutational allelic combinations after analysis (i.e., selecting events with only one to three mutated alleles of a tetraploid potato) or by selecting in-frame or tailored mutations with specific amino acid deletions or changes. DSB- and NHEJ-based mutations have limitations since they are randomly composed, often small inserts or deletions (indels). Furthermore, exact design of the outcome of a mutation would further improve the specificity and enhance the use of the methods to tailor regulating elements or target encoding active sites, or to add a stop codon to secure loss of enzymatic function. The development of CRISPR-based methods that result in tailored point mutations is ongoing for potato, but they currently have a lower frequency of induced mutations. In this context, DSBs in combination with HR [3], using a template for designed repair, as well as BE [6,13] and **prime editing (PE)** [14] have been published. Further development of the methods will be important tasks to broaden the use of genome editing in potato from mainly providing knockouts to also being used for designed knock-ins and base substitutions.

screened for. In one of the primary studies, point mutations were induced in ALS by **homologous recombination (HR)**, where the TALEN- and CRISPR-Cas9 components and a template for repair were expressed using a geminivirus replicon system and *Agrobacterium*-mediated transformation [3]. Increased tolerance to the herbicide was found in both TALEN and CRISPR lines treated with imazamox, which was calculated based on tissue weight compared with wild-type plants, 4 weeks post-spraying.

Potato with improved starch quality

Native starch comprises two glucose polymers, an essentially long-chain linear amylose and a highly branched amylopectin. For potato, no genetic variation through spontaneous mutations has been found that would affect the ratio of the two molecules of approximately 1:4. CRISPR-Cas9 based mutations have been induced in starch biosynthetic genes, altering the ratio of the molecules and the quality of the starch. A pure amylopectin starch has advantages over native starch as it facilitates more sustainable downstream processing with decreased use of chemicals and energy. For the development of this trait, a *GRANULE BOUND STARCH SYNTHASE* (*GBSS*) gene was mutated using a transgene-free DNA method on potato protoplast [4]. During the study, it was found that a fraction of the events contained undesired inserts from the DNA vector used. The introduction of the same trait was soon repeated with CRISPR-Cas9 components delivered as **ribonucleoprotein (RNP)** complexes, which eliminated the insertion of unintended DNA when synthetically produced RNA was used [5]. Another strategy to develop an amylopectin potato was described soon afterwards, where the use of **base editing (BE)** to alter the catalytic region of the GBSS enzyme was found successful [6]. Amylopectin starch is most likely the most progressed trait developed with genome editing in potato, and non-transgenic amylopectin lines are currently grown in the field for selection and seed multiplication.

A starch with long glucose chains and a low degree of branching, such as amylose, can be classified as resistant starch and can yield a low **glycemic index** in food products, as well as having improved film-forming properties compared with native starch. The degree of branching and chain-length distribution of starch was altered using CRISPR-Cas9-induced mutations in one or both of two *STARCH BRANCHING ENZYME* genes (*SBE1* and *SBE2*). In a first study, both stable transformation and PEG-mediated protoplast transfection were used [7]. Mutations in the *SBE2* gene alone gave a 15% decrease in the degree of branching, while tetra-allelic mutations in both *SBE1* and *SBE2* resulted in a more significant decrease, down to half that of wild-type starch. In a subsequent study, RNP transfection of protoplasts was used, and regenerated potato genotypes containing starch without any detectable branching and essentially free of amylopectin when all eight alleles had indels introduced [8]. In both studies, at least one allele in one or both of the genes was an in-frame mutation, and complete loss of both enzymes was speculated to detrimentally reduce vitality.

Potato with improved tuber quality

Potatoes are most often stored cold for increased shelf life, which induces starch degradation and sweetening. When tubers are processed at high temperatures, reducing sugars reacts with free amino acids causing browning and form the carcinogenic compound acrylamide. Using **transient expression** of TALEN in isolated protoplasts, mutations were targeted to a vacuolar invertase gene (*Vlnv*) that catalyzes the splitting of sucrose into fructose and glucose. In chips produced from potatoes harvested from a tetra-allelic mutated line, the acrylamide levels were found to be 73% reduced compared with wild type [9].

Enzymatic browning is a problem for potato incurred during harvest and post-harvest handling, resulting in the loss of nutritional quality, taste, and texture. The dark-colored pigments are products of an oxidation reaction catalyzed by POLYPHENOL OXIDASE (PPO) enzymes.

Box 2. Breeding strategies to increase late blight resistance

Late blight, caused by the oomycete *Phytophthora infestans*, is a devastating disease and requires the most extensive and costly pest management program during potato cultivation. Introducing resistance to *P. infestans* is therefore often high on the wish list in potato breeding. In Figure I, examples of different breeding strategies and potential future strategies to increase resistance, like traditional crossbreeding, traditional genetic modification, and the evolving genome editing methods and strategies are shown.

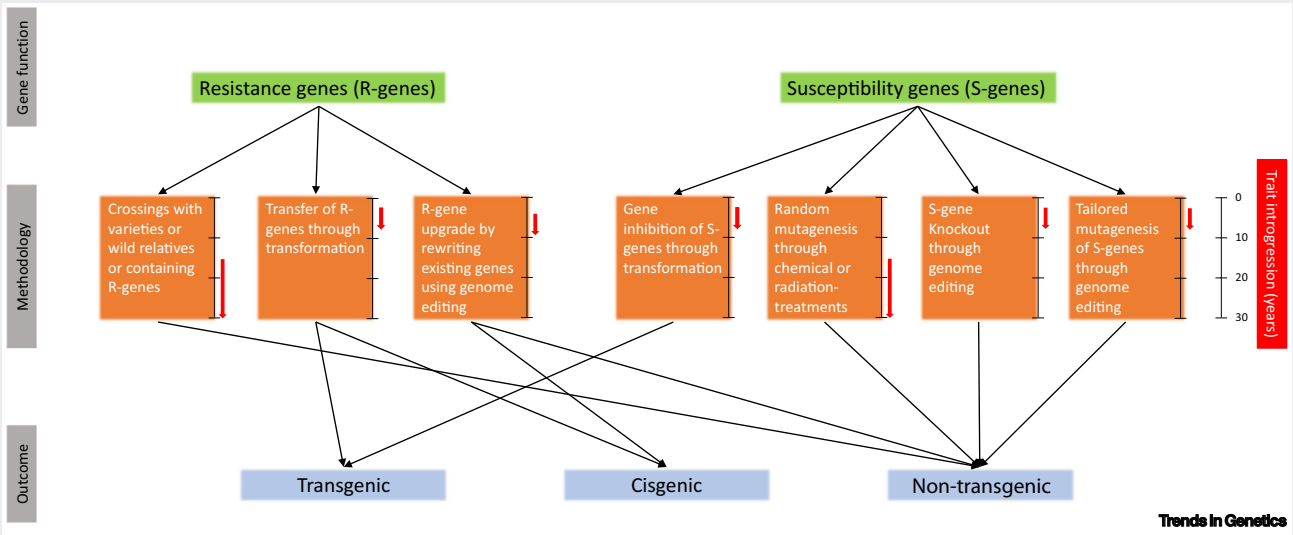

Figure I. Examples of breeding strategies to increase late blight resistance in potato. Two main target strategies are displayed – adding or modulating resistance genes (R-genes) and knocking out, inhibiting, or modulating susceptibility genes (S-genes) – that might be used both individually and in combination to make the plants more resistant to the disease. Based on the breeding method used, the times for trait introgression differ substantially and the outcome will be a **transgenic**, non-transgenic, or **cisgenic** plant that might be differently regulated based on the technique and where in the world it will be cultivated.

The PPO-encoding genes belong to a family that is differently expressed in various tissues. Currently, browning can be managed using chemical or physical post-harvest treatments. To address this problem, CRISPR-Cas9 through RNP and protoplast transfection was used to induce mutations in a *PPO2* gene, encoding the protein accountable for the largest portion of PPO activity in tubers [10]. By inducing tetra-allelic mutations, enzymatic browning was decreased to approximately 25% of the wild type.

Sterol glycoalkaloids (SGAs) are toxic compounds synthesized in the entire potato plant, and for safety reasons an upper limit of 200 mg/kg potato tubers (fresh weight) has been set in many countries. The basic level of SGAs varies among varieties and can increase through biotic stress or harvest and post-harvest conditions, such as wounding and light exposure of tubers. By inducing mutations in a *STEROL SIDE CHAIN REDUCTASE 2* (*SSR2*) gene, using *Agrobacterium*-mediated transformation of a TALEN [11] and later a CRISPR-Cas9 expression cassette [12], SGA levels were found to be reduced. In the TALEN study, a transformant with tetra-allelic mutations had reduced SGA levels to 10% of the wild type in leaves. In the CRISPR study, the effects differed between tissues and events analyzed but were most evident in leaves, where a decrease of up to 56% was observed. The majority of the events also had lower levels in the potato tuber peel of up to 46%.

Concluding remarks

Using genome editing methods such as TALEN and CRISPR, genetic variation can today be efficiently introduced in potato where natural variation is insufficient. This has now enabled the cumbersome potato breeding process to be accelerated in improvements of specific traits in potato in an already good genetic background. The examples given here are traits with health benefits, such as lowering toxic compounds and improving potato, and starch quality, or traits that tackle environmental concerns, like food loss, use of pesticides, and the use of chemicals in downstream processes. Although the traits described here are numerous and broad in application, they represent only a first few examples of what these techniques can do to contribute to future safe and sustainable food production.

Declaration of interests

No interests are declared.

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