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Genetic improvement of horticultural crops mediated by CRISPR/Cas: a new horizon of possibilities

Alessandra Koltun[1]; Lígia Erpen-Dalla Corte[2]; Liliane Marcia Mertz-Henning[3]; Leandro SA Gonçalves[2]

[1]Universidade Estadual de Maringá (UEM), Maringá-PR, Brazil; koltun@usp.br; [2]Universidade Estadual de Londrina (UEL), Londrina-PR, Brazil; ligia_erpen@yahoo.com.br, leandrosag@uel.br (corresponding author); [3]Embrapa Soja, Londrina-PR, Brazil; liliane.henning@embrapa.br

ABSTRACT

The burden of the current global challenge involving food security lies in the need to improve crop production. In this regard, biotechnology stands out as an essential tool to generate plants able to cope with pests, diseases, and harsh climatic conditions, and more efficient in the use of natural resources. An advanced approach to create genetic variability in a precise and targeted way, the genome-editing technique CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR associated proteins), has drawn the attention of breeders. The genome editing CRISPR/Cas system relies on a guiding RNA that directs a nuclease to generate a double-strand break (DSB) at a target DNA, activating the cell repair systems and eventually leading to deletions or insertions of nucleotides. Therefore, CRISPR/Cas is a toolbox to achieve many goals, from basic science investigations to the development of crops with improved agronomic traits, with potential to bring innovative solutions to food production. The CRISPR/Cas system has been applied in a large number of plants, including some horticultural species. In this review, we present details of the CRISPR/Cas natural and artificial systems, its possibilities as a biotechnological tool, advantages over other breeding techniques, regulatory issues, and its applicability in horticultural crops, as well as future challenges.

Keywords: Genome editing, plant breeding, biotechnology, induced mutations, non-transgenic.

RESUMO

Melhoramento genético de hortaliças mediado por CRISPR/Cas: uma nova gama de possibilidades

O grande desafio para garantir a segurança alimentar global está na necessidade de aumentar a produção agrícola. Neste contexto, a biotecnologia destaca-se como uma ferramenta importante para gerar plantas geneticamente melhoradas, com maior resistência/tolerância a pragas, doenças e condições climáticas adversas, que utilizem de forma eficiente os recursos naturais. Uma abordagem avançada para a geração de variabilidade genética de maneira precisa e direcionada tem chamado a atenção dos melhoristas, a técnica de edição genômica CRISPR/Cas (repetições palindrômicas curtas agrupadas e regularmente interespaçadas/proteínas associadas ao CRISPR). O sistema de edição genômica CRISPR/Cas é formado por um RNA guia que direciona uma nuclease para gerar cortes específicos no DNA alvo. Esta ruptura ativa os sistemas de reparo celular e, eventualmente, leva a deleções ou inserções de nucleotídeos. Assim, a tecnologia CRISPR/Cas pode ser aplicada tanto na pesquisa científica básica, quanto no desenvolvimento de culturas com características agronômicas melhoradas, com o potencial de trazer soluções inovadoras para a agricultura. Esta técnica tem sido aplicada em um grande número de culturas agrícolas, incluindo algumas hortaliças. Nesta revisão, apresentamos detalhes do sistema natural e artificial CRISPR/Cas, suas possibilidades como ferramenta biotecnológica, vantagens sobre outras abordagens de melhoramento genético, questões regulatórias e sua aplicabilidade nas hortaliças, bem como desafios futuros.

Palavras-chave: Edição genômica, melhoramento genético de plantas, biotecnologia, mutações induzidas, não-transgênico.

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Horticultural crops, which comprise vegetables, fruits, flowers, as well as medicinal and aromatic plants, are an essential part of agriculture production. In particular, fruits and vegetables are crucial to a balanced diet, providing energy and essential vitamins and minerals that are beneficial to human health. The burden of the current global challenge involving food security lies in the need to improve horticultural crop production (Van den Broeck & Maertens, 2016). Thus, the development of cultivars with enhanced tolerance to biotic and abiotic stresses accounts for the primary targets of genetic breeding programs. Furthermore, the goals in plant breeding have been expanded to the increasing of shelf-life, to reduce post-harvest losses, and the improvement of fruit quality traits linked explicitly to consumer preferences, such as flavor and nutraceutical compounds (Hansson *et al.,* 2018; Neves *et al.,* 2018).

Over the years, conventional plant breeding has been proved successful in addressing several of these critical characteristics. However, in some cases, conventional strategies have limited application due to non-availability

of target alleles in the germplasm of a particular species. In this case, transgenic technology represents a potential tool for genetic enhancement since it allows the introduction of specific genes which may come from a non-crossable-plant species or a non-plant-organism (Anami *et al.*, 2013). A large number of transgenic horticultural crops have been developed in the last 30 years carrying relevant modifications (Parmar *et al.*, 2017). Although the use of genes from different species is still an important tool for developing improved crops for a variety of traits, a more advanced and precise approach has drawn the attention of the breeders: the genome editing using engineered nucleases. The clustered regularly interspaced short palindromic repeats (CRISPR) associated proteins (CRISPR/Cas) system is the most recent tool developed for this purpose, allowing researchers to delete, replace, or insert specific sequences in a targeted location of the genome to generate new valuable traits with potential to bring innovative solutions to agriculture (Cardi, 2016; Liu *et al.*, 2016).

Genome editing

Over the years, artificial genetic manipulation has been used to unravel the function of genes and their regulation in metabolic pathways to create a phenotype of interest. Initially, genetic modifications were artificially performed mainly by mutagenesis with radiation or chemical agents, followed by the identification of individuals presenting the desired or the aberrant phenotypes. Although this method has contributed to the study and understanding of several cellular and molecular processes (Chen *et al.*, 2012; Haydon *et al.*, 2013), it requires the screening of thousands of individuals carrying random mutations. Consequently, it is challenging to apply it in plant breeding programs, especially those involving quantitative agronomic traits, such as yield, nutritional quality, and resistance and/or tolerance to biotic and abiotic stresses.

Advances in science created the opportunity to drive mutations at specific sites in the genome, saving time and the laborious work of inducing and screening random mutations. This great challenge was surpassed in the 90s when the first tools emerged, relying on engineered nucleases linked to components capable of recognizing DNA sequences. The first technique successfully used for this purpose was the zinc finger nuclease (ZFN), which identify specific DNA sequences through protein-DNA interactions and guide an associated endonuclease (commonly Fok I), allowing the manipulation of particular targets in the genome (Durai *et al.*, 2005). Later, a similar genome editing tool was developed, based on transcription effectors identified in the plant pathogen *Xanthomonas* spp., called transcription activator-like effectors (TALEs) (Bogdanove *et al.*, 2010). These effectors can be customized to recognize and bind to specific DNA guiding endonucleases to induce site-specific editions in the genome in a complex system called TAL effector nucleases (TALENs) (Bogdanove & Voytas, 2011). Although the ZFN and TALENs technologies have been used for genome editing in several organisms, the high cost and complexity of synthesizing DNA-binding proteins have limited their use for the study and genetic improvement of plants.

The most recent genome editing tool is the CRISPR/Cas system, identified as part of the immune mechanism against exogenous DNA in bacteria and Archaea. CRISPR/Cas uses small sequences of non-coding RNAs to guide nucleases to cleave a target DNA (Horvath & Barrangou, 2010) or a target RNA, as recently discovered (Wolter & Puchta, 2018). By using RNA as guiding molecules, this technique dispenses the laborious and expensive step of building and optimizing complex proteins (such as ZFN and TALEs) for DNA recognition, representing a more flexible and viable tool for genome manipulation (Song *et al.,* 2016). Compared to other genome editing strategies, the CRISPR/Cas technique is more straight forward, more cost-effective, precise and is highly efficient even at multiplex genome editing (Wang *et al.*, 2018). Therefore, the innumerable advantages of this new technology have gradually made previous tools, such as ZFN and TALENs, become increasingly obsolete.

The CRISPR/Cas system

The opportunity to exploit the CRISPR/Cas system as a biotechnological tool came from a deeper understanding of the underlying molecular mechanisms of the natural process in prokaryotes, specifically of the CRISPR/Cas9 system from *Streptococcus pyogenes*. This microbial adaptive immune system mediates defense against foreign genetic elements through three main steps: immunization, expression, and interference (Bhaya *et al.*, 2011).

During the immunization step, the host incorporates small sequences of the invader DNA (ranging from 21-48 bp) to a specific region of its own genome, the CRISPR locus, in the form of spacers between short palindromic repeats. The recognition of the region to be incorporated as a spacer depends on the presence of small conserved nucleotide sequences (2-3 specific nucleotides), called adjacent protospacer motifs (PAMs), which represent the anchoring site of the nuclease and determine the cleavage point of the target DNA (Mojica *et al.*, 2009). The expression of these regions results in small non-coding RNAs, called CRISPR-RNAs (crRNAs) and trans-activating crRNA (tracrRNA) (Makarova *et al.*, 2011). The tracrRNA participates in the maturation process of the crRNAs, forming a two-RNA structure that guides Cas9 to promote double-strand breaks (DSBs) in the foreign DNA, inactivating the genetic material of the invader at the interference stage (Deltcheva *et al.*, 2011).

Thus, in summary, the CRISPR/Cas system allows the host to construct a "library" containing an array of small fragments of foreign DNA that have previously invaded the cell, which prevents future infections by cleaving exogenous genetic materials that include library-like fragments mediated by the action of endonucleases. Briefly, it relies on two essential components: the nuclease Cas9 and a guide, which is composed of two RNA sequences. Among the adaptions of the natural process to form the biotechnological

tool is the single guide RNA (sgRNA), a combination of the dual-tracrRNA:crRNA (type II system), which simplifies the system (Jinek *et al.*, 2012). Moreover, the ability to drive the Cas9 nuclease to the target site comes from the specificity of a sequence of about 20 bp located in the 5' portion of the guide RNA, which has homology to the sequence of interest (Cong *et al.*, 2013). By manipulating this 20 bp sequence, it is possible to create specific sgRNA molecules for different targets in the genome, which makes the CRISPR/Cas technique an extremely versatile tool, applicable to a wide range of species.

As mentioned above, an essential requirement for the functionality of the system is that the sgRNA must have homology to a fragment of DNA that is adjacent to the anchoring site of the endonuclease, a small conserved nucleotide sequence (2-3 nucleotides), the PAM (Mojica *et al.*, 2009). Interestingly, the sequence that determines PAM varies for homologous Cas present in different organisms. The Cas9 of *S. pyogenes* (SpCas9), for example, recognizes the sequence PAM 5'-NGG-3' and performs a blunt DSB at approximately three nucleotides upstream of the PAM sequence (Jinek *et al.*, 2012). Although Cas9 of *S. pyogenes* is the most widely used wild endonuclease, homologous Cas9, such as *Staphylococcus aureus* (SaCas9), are important since they represent an extension of the range of PAM sequences that can be used in the CRISPR/Cas system. (Kleinstiver *et al.*, 2015).

Furthermore, although the wild Cas9 nuclease of *S. pyogenes* proved to be functional for editing the genome of plants and other eukaryotes, several research groups performed optimizations on the original Cas9 codons, aiming for greater efficiency in the species studied, for instance monocots or dicots (Wang *et al.*, 2014, Michno *et al.*, 2015). The addition of the nuclear localization signal (NLS) to the enzyme sequence in the transformation vector was another adaptation to the editing of eukaryotic genomes (Michno *et al.*, 2015). These signals ensure that after translation in the cytoplasm of the cell, the nucleases are transported to their site of action, the nucleus.

Recently, the diversity of this system has been investigated. Researchers screened for other nuclease families with features distinct from Cas9 and found some efficient enzymes, such as Cpf1 and Cms1 (CRISPR from *Microgenomates* and *Smithella*). These nucleases utilize a T-rich and AT-rich PAM, respectively, mediating robust DNA cleavage via a staggered (sticky-end) DNA DSB (Zetsche *et al.*, 2015). Identifying these variant nucleases and distinct mechanisms of interference broaden our understanding of the CRISPR/Cas toolbox, increases the diversity of options available to researchers and advances genome editing applications.

In summary, billions of years of evolution have developed and improved a system that cuts prokaryotic DNA in a site-specific manner. Researchers investigated and adapted it to become a biotechnological tool for editing the genome of many species, including eukaryotes. After the DSB at the genome target region caused by CRISPR/Cas, natural repair mechanisms present in the cell are recruited to the cleavage region, the main ones being the non-homologous end joining (NHEJ), which is prone to errors, and the better, but more complicated, homology-directed repair (HDR) (Dexheimer, 2013).

During the NHEJ repair, the ends of the break are ligated and small insertions and deletions (Indels) at this site may occur, leading to imperfect repair and various mutations. These modifications may result in changes in the reading frame during translation of the mRNA and in the onset of a premature stop codon, culminating in the knockout of the gene of interest or loss of functionality of the encoded protein. The NHEJ repair system is the primary mechanism used in genome editing strategies in plants.

The HDR mechanism maintains the genome integrity by using a model DNA to correct a DBS, a sister chromatid in dividing cells, for instance (Escribano-Díaz *et al.*, 2013). In genetic manipulation, a donor DNA homologous to the site of cleavage is provided to the cell. However, it may contain a new sequence between the homology borders, which is going to be copied by the cell. This action represents a highly specific genome editing technique, wherein the new sequences can be stipulated, unlike the random mutations the NHEJ repair system causes. The sequences inserted can be fragments of a gene, whole genes, and site of transcription factors in the promoter region or even entire promoters. It has not been vastly used for editing plant genomes because of the low frequency of target mutation and recombination (Puchta, 2005). However, the discovery and application of new endonuclease families have increased the efficiency of the HDR pathway.

Inducing a DSB in the DNA of an organism to activate a repair pathway has a great potential application for plant breeding, such as silencing of undesired native genes; alteration of the nucleotide sequence encoding amino acids, aiming at improving a protein activity (the transfer of complete or partial gene sequences or entire allele swapping); and fine-tune expression of native genes of interest acting upon the promoter region (repressing or adding artificial transcription factors, for example). Therefore, not only can native genes be modified and manipulated but novel traits can be inserted as well.

After defining the type of endonuclease and the approach of the system (NHEJ or HDR), plant genomic editing via CRISPR/Cas can be divided into four main steps: 1) selection of the target regions of the sgRNA (that must be unique in the genome and adjacent to the PAM specific to the nuclease chosen); 2) design and construction of CRISPR/Cas transformation vectors containing the sgRNAs, the Cas nuclease and the donor DNA (in the case of HDR); 3) transformation of explants and regeneration of transformed plants, and 4) screening of mutations in T0 plants (Liang *et al.*, 2016).

Among the prerequisites to perform genomic editing, the target organism must have its genome sequenced, to identify specific sites in the sgRNA, thus avoiding modifications at undesired

sites (off-target edition).There are some horticultural crops with sequenced genomes of reference available, such as tomato (*Solanum lycopersicum*) (The Tomato Genome Consortium, 2012), pepper (*Capsicum annuum*) (Kim *et al.*, 2014), cucumber (*Cucumis sativus*) (Huang *et al.*, 2009), sugar beet (*Beta vulgaris*) (Dohm *et al.*, 2014), potato (*Solanum tuberosum*) (The Potato Genome Sequencing Consortium, 2011) among others. The screening of the plant genome avoids designing sgRNAs that bind to DNA sequences that present homology to other regions, which would lead to DSBs at sites other than the target, eventually leading to aberrant or unwanted phenotypes. In addition to specific sgRNAs, an efficient method for introducing DNA into plant cells, competence for regeneration (well-established protocol to generate whole plants from the genetically modified cells cultivated *in vitro*) and a suitable selection system are required. Several horticultural crops still lack these basic requirements, which represent the greatest challenge for the CRISPR/Cas tool to be applied. Further studies are necessary.

The CRISPR/Cas system has some advantages when compared to the genetic engineering tool commonly used, the transgenic. It is important to highlight that CRISPR/Cas enables direct DNA insertion at strategic locations of the genome, presenting rates of expression higher than that observed for transgenic, that induces random insertions. Besides, the CRISPR/Cas expression cassette may be introduced in one chromosome and perform the edition in another. Therefore, traits can segregate in cross-breeding, generating non-transgenic mutant plants (that no longer has the CRISPR/Cas locus) (Figure 1) (Chandrasekaran *et al.*, 2016). Besides, a recent study has shown the possibility of inducing modifications in the genome using the CRISPR/Cas9 technology transiently expressed in calluses, which were later used to regenerate whole plants, edited and non-transgenic. The CRISPR/Cas9 system is delivered as a protein-RNA complex degraded after edition and not as a DNA sequence permanently introduced in the plant genome (Zhang *et al.*, 2016). As such, this is a tremendous breakthrough for perennial crops, wherein the time range of each generation is a drawback and does not allow several crosses.

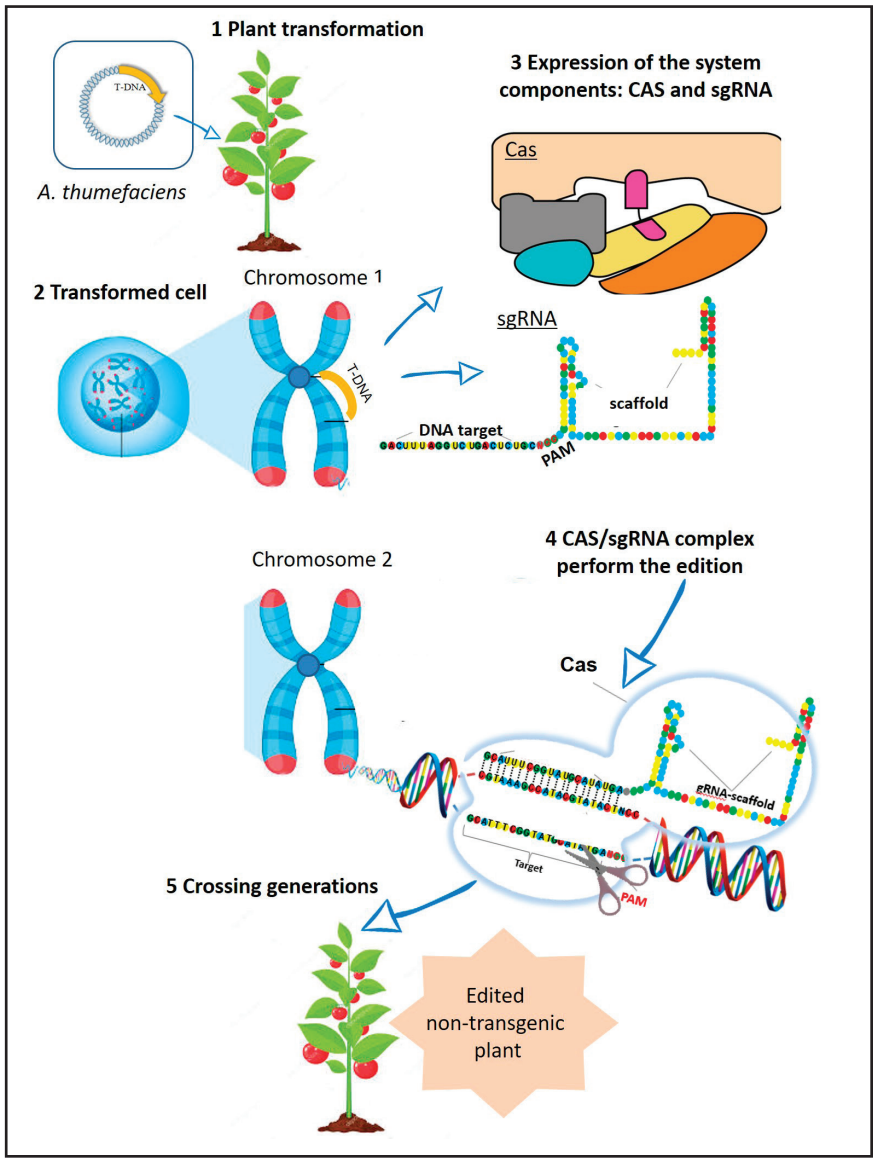

Figure 1. Schematic representation of the process to develop an edited non-transgenic plant. Step 1) Plant transformation, introduction of the expression cassette containing the sequences of a Cas nuclease and a sgRNA via *Agrobacterium tumefaciens*; Step 2) Selection of the transformed cells that received the system sequence at chromosome 1 (randomly); Step 3) Expression of the components Cas and SgRNA of the system in the cytoplasm; Step 4) Cas and the sgRNA form a complex and are directed to the nucleus where it performs a DBS at the DNA target in chromosome 2; Step 5) chromosome independent segregation in the crossing generations (2nd Mendel's law) makes it possible to select plants with chromosome 1 without the CRISPR/Cas insertion and chromosome 2 carrying the edition. Londrina, UEL, 2018.

To generate an edited non-transgenic plant becomes even more critical when it comes to species used directly as human food, like most vegetables and fruits. Although the world uses transgenic crops for more than 30 years, population acceptance of genetically modified organisms (GMOs) is still a substantial impediment to the commercialization of these products (Bradford *et al.*, 2005). Furthermore, the development and release to the market of transgenic crops require very high investments of both time and resources, amounting to an estimate of $136 million, from the discovery of the trait to the authorization and commercial launch (McDougall,

2011). From this total, the regulatory testing and the registration processes demand about 26% ($35.1 million) and 5.5 years. The high cost of the whole process limits it to companies with the required resources, and the few ones capable of developing transgenic crops focus on a few economically valuable traits.

With minor exceptions, the GMOs presently in use are cultivars of crops used for feed, consumable oil, and fiber production (maize, soybean, canola, and cotton), developed by multinational companies for farmers in the United States and Canada, which were later adapted to some other countries (Brazil, Argentina, India). The vast majority presents the same two functional traits (herbicide and insect tolerance), both introduced in 1996 (Pingali & Feder, 2017). Therefore, the high cost of developing a crop with a biotechnological advantage narrows the possibility of more public and private companies to generate GMOs addressing the needs of developing country farmers or focused on other traits such as nutritional quality.

Although the use of genes from different species is still an essential tool in developing improved plants for a variety of traits, the high cost of regulation of a transgenic crop and population constraints hinders the generation and commercialization of these products. The possibility of using CRISPS/Cas strategies that generate non-transgenic mutants would save millions of dollars and years of work, circumventing the need to regulate a transgenic. Such situation would benefit researchers when addressing agricultural challenges and also the public sector, universities and other parties which would have more chances of developing biotechnological assets. At the same time, it would increase public acceptance, allowing advances in food production and contributing to food security.

These new breeding technologies (NBTs), which include the CRISPR/Cas system, have required special attention under the regulatory point of view and have been discussed and established in the adopting countries such as Brazil, Argentina, China, USA, and Canada. As mentioned above, some of the genetic manipulations may not be considered as transgenic, since the technique can induce new genetic variations without leaving traces of genes from another species in the final product, generating improved plants identical to the original plants, except for the targeted mutation. It is crucial to implement similar criteria in the analysis and deliberation of products to avoid commercialization impediments.

CRISPR/Cas in horticultural crops

Genome editing using the CRISPR/Cas system in plants was first reported in 2013, and since then it has received extensive attention (Li *et al.*, 2013; Nekrasov *et al.*, 2013; Shan *et al.*, 2013). A complete search on Scopus and Web of Science databases, using the following query: "CRISPR" and "plants" showed several results (Figure 2). It can be observed that in a short time, numerous examples of studies involving the CRISPR system in plants were published, demonstrating its potential as a genomic tool for plant biology studies and crop improvement.

Among the studies, there are many reports of successful gene editing by CRISPR/Cas9 in horticultural crops (Table 1). Since it is still a very recent method, many authors have sought to introduce mutations in genes that would result in a distinctive and immediately recognizable phenotype, to test and optimize the efficacy of the CRISPR/Cas9 technique. Klimek-Chodacka *et al.* (2018) directed mutations in the carrot *F3H* gene, essential for anthocyanin biosynthesis in purple-colored carrot, resulting in the regeneration of discolored carrot calli. Tian *et al*. (2017) selected the *ClPDS* gene required for chlorophyll biosynthesis, and watermelon mutant plants exhibited evident albino phenotype. The gene *SLAGO7* regulates organ polarity in tomato, and loss-of-function mutant plants had abnormal leaves - leaflets without petioles and leaves lacking laminae - early in the tissue culture phase (Brooks *et al.*, 2014). Lawrenson *et al*. (2015) generated Cas9-induced mutations in the *BolC.GA4, a* gene from *Brassica oleracea*, leading to plants with the expected dwarf phenotype associated with target gene knockout.

Genome editing has been widely used in plant research also to study gene functions and fundamental biological processes. The CRISPR/Cas9 system was applied to mutate two genes likely to be involved in auxin biosynthesis and signaling, *FveTAA1* and *FveARF8*, in wild strawberry *Fragaria vesca*. The authors generated transgenic plants harboring knockout mutations in the target genes. The arf8 mutant seedlings showed faster growth than wild-type plants, suggesting that the *FveARF8* gene plays a repressor role in auxin signaling (Zhou *et al.*, 2018). CRISPR/Cas9-engineered mutations in the tomato *SlBOP* gene caused severe inflorescence defects and allowed to

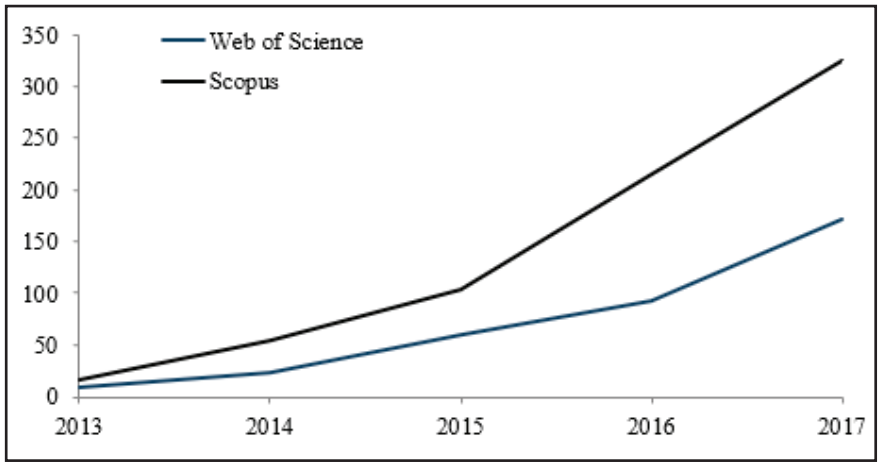

Figure 2. Number of papers reporting the use of the CRISPR/Cas system in plants from 2013 to 2017. Londrina, UEL, 2018.

Table 1. CRISPR/Cas mediated genome editing in horticultural plants. Londrina, UEL, 2018.

Crop	Target Gene	Plant phenotype	Reference
Cabbage	*BoIC.GA4.a*	Dwarf phenotype	Lawrenson *et al*. (2015)
Carrot	Flavanone-3-hydroxylase (*DcF3H*)	Blockage of the anthocyanin biosynthesis in purple-colored carrot	Klimek-Chodacka *et al*. (2018)
Cucumber	Eukaryotic translation initiation factor 4E (*eIF4E*)	Resistance to Cucumber vein yellowing virus; Potyviruses Zucchini yellow mosaic virus; Papaya ring spot mosaic virus-W	Chandrasekaran *et al*. (2016)
Potato	Granule-bound starch synthase (*StGBSS*)	Starch with reduced amylose content and increase in the amylopectin/amylose ratio	Andersson *et al*. (2017, 2018)
	16α-hydroxylation (*St16DOX*)	Complete abolition of the steroidal glycoalkaloids accumulation	Nakayasu *et al.* (2018)
Strawberry	Auxin Response Factor 8 (*FvARF8*); Auxin biosynthesis gene (*FveTAA*1)	Increasing of auxin biosynthesis leading to faster growth of seedlings	Zhou *et al.* (2018)
Tomato	ARGONAUTE7 (*SlAGO7*)	First leaves having leaflets without petioles and later-formed leaves lacking laminae	Brooks *et al.* (2014)
	Ripening inhibitor (*RIN*)	Incomplete ripening fruits with reduced red color pigmentation	Ito *et al.* (2015)
	Blade-on-petiole (*SlBOP*)	Inflorescence defects	Xu *et al.* (2016)
	Aux/IAA9 (*SlIAA9*)	Morphological changes in leaf shape and seedless fruit	Ueta *et al.* (2017)
	SlAGAMOUS-LIKE 6 (*SlAGL6*)	Production of seedless fruits with normal weight and shape under heat stress	Klap *et al.* (2017)
	Self-pruning 5G (*SlSP5G*)	Loss of day-length-sensitive flowering, enhancement of the compact determinate growth habit and early yield	Soyk *et al.* (2017)
	Mildew resistant locus (SlMlo1)	Resistance to powdery mildew	Nekrasov *et al.* (2017)
	Alcobaça *(SLALC)*	Long-shelf life	Yu *et al.* (2017)
	Glutamate decarboxylase (SlGAD2 and SlGAD3)	Fruits with increased ɣ-aminobutyric acid (GABA) content	Nonaka *et al.* (2017)
	lncRNA1459	Fruits with ripening, ethylene production, and lycopene accumulation repressed	Li *et al.* (2018)
Watermelon	Phytoene desnaturase (*ClPDS*)	Albino phenotype	Tian *et al.* (2016)

prove its dominant role in flowering and inflorescence architecture (Xu *et al.*, 2016).

Similarly, to improve the understanding of the tomato photoperiod response, Soyk *et al*. (2018) generated a loss of day-length-sensitive flowering in tomato, with mutations in the *SP5G* gene. The knockout of the *SlAGL6* gene underlined its role in facultative parthenocarpy, leading to the production of seedless tomato fruits with regular weight and shape, under heat stress (Klap *et al.*, 2017). Site-directed mutagenesis in tomato has also been used to study the regulation of ripening, wherein a series of mutations that potentially eliminate the function of a *RIN* gene (Ito *et al.*,

2015) and the *lncRNA1459* gene (Li *et al.*, 2018) resulted in mutants with deficient-ripening fruit production.

The ability to modify genomes in a site-directed manner has also been applied to develop cultivars with new traits. Parthenocarpic fruit is an attractive attribute since it allows the production of seedless fruits. Ueta *et al*. (2017) demonstrated a CRISPR/Cas9 strategy to effectively introduce mutations into *SlIAA9*, a crucial gene controlling parthenocarpy in tomato. Transgenic tomato plants of Micro-Tom and commercial cultivar Ailsa Craig, carrying bi-allelic and homozygous mutations, exhibited the typical phenotypes of parthenocarpy, described as fruit development before pollination, leading to seedless fruits. A small number of fertilized fruits developed a few seeds, which produced tomato plants exhibiting the mutations and associated phenotypes.

The long shelf life is another important characteristic in fleshy fruit that influences fruit marketability and can reduce fruit loss. For exploiting this quality attribute in tomato, the CRISPR/Cas9 system was applied for obtaining the tomato *ALC* gene replacement in the presence of the homologous repair template (replacement of thymine by adenine in position 317 of the coding sequence). The replacement efficiency was low in T0 transgenic plants, and only one individual with a heterozygous mutation was obtained, requiring further segregation to generate the homozygous mutation. In the T1 generation, it was possible to generate recessive homozygous *alc* mutants free of the CRISPR/Cas9 components which presented excellent storage performance (Yu *et al.*, 2017). Nonaka *et al*. (2017) report another study involving fruit quality in tomato. The authors increased γ-aminobutyric acid (GABA) accumulation in tomato fruit, by removing the autoinhibitory domain of *SlGAD2* and *SlGAD3* genes, through the CRISPR/Cas9 system. The consumption of GABA-enriched foods in daily life can bring anti-hypotensive effects and would be an interesting path to prevent hypertension in humans.

Steroidal glycoalkaloids (SGAs), such as α-solanine and α-chaconine, are naturally occurring toxic compounds in potato tubers that can cause a bitter taste and undesirable effects in humans when present at high levels. Nakayasu *et al.* (2018) demonstrated a CRISPR/Cas9 strategy to reduce the SGA content in potato. The authors produced transgenic hairy roots carrying multiple mutations at different sites in *St16DOX* gene, encoding a steroid 16α-hydroxylase in SGA biosynthesis. These mutations lead to complete abolition of the SGA accumulation in potato hairy roots.

The CRISPR/Cas technology has also found application in developing disease-resistant plants. A tomato variety resistant to the powdery mildew fungal pathogen *Oidium neolycopersici* was developed by creating loss-of-function mutations in the mildew resistant locus O1 (*Mlo1*), which encodes a membrane-associated protein that confers susceptibility to the fungal pathogen. Lines from the T1 generation with mutations in both alleles were fully resistant to the pathogen compared to the wild-type (Nekrasov *et al.*, 2017). Chandrasekaran *et al*. (2016) reported the generation of a cucumber with a broad virus resistance, by knocking out the cucumber *eIF4E* gene, a plant cellular translation factor essential for the Potyviridae life cycle. Homozygous T3 progeny exhibited immunity to the Cucumber vein yellowing virus (Ipomovirus) infection and resistance to the potyviruses Zucchini yellow mosaic virus and Papaya ringspot mosaic virus-W. The disease resistant tomato and cucumber plants were also transgene-free. In both instances, since the transgene Cas9/sg RNA and the gene mutation sites were at different genome locations and had independent segregation, it was possible to select non-transgenic mutants in later generations, i.e., plants containing the mutation without carrying any foreign DNA. The same strategy was used to generate long shelf life tomato lines (Yu *et al.*, 2017). These examples emphasize the considerable potential of this tool, beyond that of transgenics, since it can offer an efficient method to avoid random gene insertions throughout the genome and, more important, it allows for the possibility of removing foreign DNA, a big concern related to transgenic plants.

The value of eliminating CRISPR/Cas9 components inserted in the genome via selfing or backcrossing is more complicated in genetically complex and vegetative propagated species, such as potato. To overcome this difficulty, Andersson *et al*. (2017) demonstrated the transient transfection of the CRISPR/Cas9 construct in protoplasts isolated from tetraploid potato and, subsequently, shoot regeneration containing the desired mutations. Using this approach, the authors were able to produce a few lines lacking any DNA integration and carrying mutations in at least one allele, multiple or four alleles of the gene *StGBSS*, encoding granule-bound starch synthase. Complete elimination of the *StGBSS* enzyme function in four-allele mutated lines led to the production of starch with altered amylose synthesis and a concomitant increase in the amylopectin/amylose ratio, a desirable trait in potato tubers for culinary and industrial processes.

Another alternative to generate genome-edited plants lacking any DNA integration is delivering CRISPR/Cas9 as ribonucleoproteins (RNPs) into cells. To test this technique in potato, Andersson *et al.* (2018) employed the same previous approach (knockout of the *StGBSS* gene function) but using ribonucleoproteins for protoplast transfections rather than plasmid DNA. Briefly, the authors obtained regenerated plants with the specific desired mutations, without DNA integration, but with the advantage of a higher frequency of transgene-free mutated lines, which can reduce the size of screening populations. The development of genome-edited plants that do not possess any foreign DNA (transgene) would be an essential step to increase consumers' acceptance, as well as to reduce the cost and time for releasing new cultivars since these plants may not be subject to the current regulatory approval process applied to genetically engineered plants.

Final considerations

The CRISPR/Cas technology is a powerful tool for creating genetic variability in a precise and targeted way, representing a new era in crop breeding. The system is versatile, fast and low-cost, allowing genome editing strategies to be more accessible and efficient than other technologies (mutagenesis, ZFNs, and TALENs). Its effectiveness has already been proven in several species, many of them of commercial interest, so the speed of diffusion of the technique in the scientific and commercial environment depends more on the regulatory aspects related to biosafety. The possibility of generating an edited non-transgenic plant may facilitate the development of biotechnological crops, saving time and money and thus broadening the range of public and private companies capable of working on different species and traits, as well as increasing the acceptance biotechnological crops by the population that still has some resistance to transgenics.

Several studies with horticultural species have already been carried out as proof of concept but also addressing economically important traits, showing the applicability of the technique. Some horticultural crops still lack basic requirements for the CRISPR/Cas tool to be applied: information about the genome and established protocols for genetic transformation and *in vitro* regeneration. Therefore, expanding knowledge on horticultural crops will allow the precise targeted engineering of endogenous genetic traits and the introduction of new traits, possibly reshaping global horticulture and assuming a leading role towards food security.

Author contributions

Alessandra Koltun and Lígia Erpen-Dalla Corte participated in all stages of manuscript development, writing and reviewing. Liliane Marcia Mertz-Henning and Leandro SA Gonçalves contributed with project planning and manuscript review. All authors read and appr approved the final manuscript.

REFERENCES

ANAMI, S; NJUGUNA, E; COUSSENS, G; AESAERT, S; VAN LIJSEBETTENS, M. 2013. Higher plant transformation: Principles and molecular tools. *The International Journal of Developmental Biology* 57: 483-494.

ANDERSSON, M; TURESSON, H; NICOLIA, A; FÄLT, AS; SAMUELSSON, M; HOFVANDER, P. 2017. Efficient targeted multiallelic mutagenesis in tetraploid potato (*Solanum tuberosum*) by transient CRISPR/Cas9 expression in protoplasts. *Plant Cell Reports* 36:117-128.

ANDERSSON, M; TURESSON, H; OLSSON, N; FÄLT, AS; OHLSSON, P; GONZALEZ, MN; SAMUELSSON, M; HOFVANDER, P. 2018. Genome editing in potato via CRISPR/Cas9 ribonucleoprotein delivery. *Physiologia Plantarum* 164: 119-240.

BHAYA, D; DAVISON, M; BARRANGOU, R. 2011. CRISPR-Cas systems in bacteria and Archaea: Versatile small RNAs for adaptive defense and regulation. *Annual Review of Genetics* 45: 273-297.

BOGDANOVE, AJ; SCHORNACK, S; LAHAYE, T. 2010. TAL effectors: Finding plant genes for disease and defense. *Current Opinion in Plant Biology* 13: 394-401.

BOGDANOVE, AJ; VOYTAS, DF. 2011. TAL effectors: Customizable proteins for DNA targeting. *Science* 333: 1843-1846.

BRADFORD, KJ; VAN DEYNZE, A; GUTTERSON, N; PARROTT, W; STRAUSS, SH. 2005. Regulating transgenic crops sensibly: lessons from plant breeding, biotechnology and genomics. *Nature Biotechnology* 23: 439.

BROOKS, C; NEKRASOV, V; LIPPMAN, ZB; VAN ECK, J. 2014. Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-Associated9 System. *Plant Physiology* 166: 1292-1297.

CARDI, T. 2016. Cisgenesis and genome editing: Combining concepts and efforts for a smarter use of genetic resources in crop breeding. *Plant Breeding* 135: 139-147.

CHANDRASEKARAN, J; BRUMIN, M; WOLF, D; LEIBMAN, D; KLAP, C; PEARLSMAN, M; SHERMAN, A; ARAZI, T; GAL-ON, A. 2016. Development of broad virus resistance in non-transgenic cucumber using CRISPR-Cas9 technology. *Molecular Plant Pathology* 17: 1140-1153.

CHEN, JH; JIANG, HW; HSIEH, EJ; CHEN, HY; CHIEN, CT; HSIEH, HL; LIN, TP. 2012. Drought and salt stress tolerance of an Arabidopsis glutathione S-transferase U17 knockout mutant are attributed to the combined effect of glutathione and abscisic acid. *Plant Physiology* 158: 340-51.

CONG, L; RAN, FA; COX, D; LIN, S; BARRETTO, R; HSU, PD; WU, X; JIANG, W; MARRAFFINI, LA. 2013. Multiplex Genome Engineering Using CRISPR/Cas Systems. *Science* 339: 819-823.

DELTCHEVA, E; CHYLINSKI, K; SHARMA, CM; GONZALES, K; CHAO, Y; PIRZADA, ZA; ECKERT, MR; VOGEL, J; CHARPENTIER, E. 2011. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. *Nature* 471: 602-607.

DEXHEIMER T. 2013. DNA repair pathways and mechanisms. In: MATHEWS, L; CABARCAS, S; HURT, E (eds). *DNA Repair of Cancer Stem Cells*. Springer, Dordrecht. p.19-32. DOI: 10.1007/978-94-007-4590-2_2

DOHM, JC; MINOCHE, AE; HOLTGRÄWE, D; CAPELLA-GUTIÉRREZ, S; ZAKRZEWSKI, F; TAFER, H. 2014. The genome of the recently domesticated crop plant sugar beet (Beta vulgaris). Nature 505 (7484): 546-549.

DURAI, S; MANI, M; KANDAVELOU, K; WU, J; PORTEUS, MH; CHANDRASEGARAN, S. 2005. Zinc finger nucleases: custom-designed molecular scissors for genome engineering of plant and mammalian cells. *Nucleic Acids Research* 33: 5978-5990.

ESCRIBANO-DÍAZ, C; ORTHWEIN, A; FRADET-TURCOTTE, A; XING, M; YOUNG, JTF; TKÁČ, J; COOK, MA; ROSEBROCK, AP; MUNRO, M; CANNY, MD; XU, D; DUROCHER, D. 2013. A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. *Molecular Cell* 49: 872-883.

HANSSON, SO; AMAN, P; BECKER, W; KONING, D; LAGERKVIST, CJ; LARSSON, I; LEHRMAN A; RISÉRUS, U; STYMNE, S. 2018. Breeding for public health: A strategy. *Trends in Food Science & Technology* 76: 481-486.

HAYDON, MJ; MIELCZAREK, O; ROBERTSON, FC; HUBBARD, KE; WEBB, AR. 2013. Photosynthetic entrainment of the *Arabidopsis thaliana* circadian clock. *Nature* 502: 689-692.

HORVATH, P; BARRANGOU, R. 2010. CRISPR/Cas, the immune system of bacteria and archaea. *Science* 327: 167-170.

HUANG, SW; LI, RQ; ZHANG, ZH; LI, L; GU, XF; FAN, W; LUCAS, WJ; WANG, XW; XIE, BY. 2009. The genome of the cucumber, *Cucumis sativus* L. *Nature Genetics* 41: 1275-U1229.

ITO, Y; YOKOI, N; ENDO, M; MIKAMI, M; TOKI, S. 2015. CRISPR/Cas9-mediated mutagenesis of the RIN locus that regulates tomato fruit ripening. *Biochemical and Biophysical Research Communications* 6: 76-82.

JINEK, M; CHYLINSKI, K; FONFARA, I; HAUER, M; DOUDNA, JA; CHARPENTIER, E. 2012. A Programmable dual-RNA – guided DNA endonuclease in adaptive bacterial immunity. *Science* 337: 816-822.

KIM, S; PARK, M; YEOM, SI; KIM, YM; LEE, JM; LEE, HA; SEO, E; CHOI, J; CHEONG, K; KIM, K; JUNG, K; LEE, G; OH, S; BAE, C; KIM, S; LEE, H; KIM, S; KIM, M; KANG, B; CHOI, D. 2014. Genome sequence of the hot pepper provides insights into the evolution of pungency in Capsicum species. Nature Genetics 46 : 270–278. DOI: 10.1038/ng.2877.

KLAP, C; YESHAYAHOU, E; BOLGER, AM; ARAZI, T; GUPTA, SK; SHABTAI, S; USADEL, B; SALTS, Y; BARG, R. 2017.

Tomato facultative parthenocarpy results from SIAGAMOUS-LIKE 6 loss of function. *Plant Biotechnology Journal* 15: 634-647.

KLEINSTIVER, B; PREW, MS; TSAI, SQ; TOPKAR, VV; NGUYVEN, NT; ZHENG, Z; GONZALES, APW; LI, Z; PETERSON, RT; YEH, JJ; ARVEE, MJ; JOUNG, JK. 2015. Engineered CRISPR-Cas9 nucleases with altered PAM specificities. *Nature* 523: 481-485.

KLIMEK-CHODACKA, M; OLESZKIEWICZ, T; LOWDER, LG; QI, Y; BARANSKI, R. 2018. Efficient CRISPR/Cas9-based genome editing in carrot cells. *Plant Cell Reports* 37: 575-586.

LAWRENSON, T; SHORINOLA, O; STACEY, N; LI, C; ØSTERGAARD, L; PATRON, N; UAUY, C; HARWOOD, W. 2015. Induction of targeted, heritable mutations in barley and *Brassica oleracea* using RNA-guided Cas9 nuclease.*Genome Biology* 6: 258.

LI, JF; NORVILLE, JE; AACH, J; MCCORMACK, M; ZHANG, D; BUSH, J; CHURCH, GM; SHEEN, J. 2013. Multiplex and homologous recombination-mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and Cas9. *Nature Biotechnology* 31: 688-691.

LI, R; FU, D; ZHU, B; LUO, Y; ZHU, H. 2018. CRISPR/Cas9-mediated mutagenesis of lncRNA1459 alters tomato fruit ripening. *The Plant Journal* 94: 513-524.

LIANG, Z; ZONG, Y; GAO, C. 2016. An efficient targeted mutagenesis system using CRISPR/Cas in Monocotyledons. *Current Protocols in Plant Biology* 1: 329-344.

LIU, D; HU, R; PALLA, KJ; TUSKAN, GA; YANG, X. 2016. Advances and perspectives on the use of CRISPR/Cas9 systems in plant genomics research. *Current Opinion in Plant Biology* 30: 70-77.

MAKAROVA, KS; GRISHIN, NV; SHABALINA, SA; WOLF, YI; KOONIN, EV. 2011. A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action. *Biology direct* 1: 7.

MCDOUGALL, P. 2011. *The cost and time involved in the discovery, development, and authorization of a new plant biotechnology-derived Trait.* A consultancy study for crop life international. Available at http://www.croplife.org/PhillipsMcDougallStudy. Accessed June 15, 2018.

MICHNO, JM; WANG, X; LIU, J; CURTIN, SJ; KONO, TJY; STUPAR, RM. 2015. CRISPR/Cas mutagenesis of soybean and *Medicago truncatula* using a new web-tool and a modified Cas9 enzyme. *GM Crops & Food* 6: 243-252.

MOJICA, FJM; DÍEZ-VILLASEÑOR, C; GARCÍA-MARTÍNEZ, J; ALMENDROS, C. 2009. Short motif sequences determine the targets of the prokaryotic CRISPR defense system. *Microbiology* 155: 733-40.

NAKAYASU, M; AKIYAMA, R; LEE, HJ; OSAKABE, K; OSAKABE, Y; WATANABE, B; SUGIMOTO, Y; UMEMOTO, N; SAITO, K; MURANAKA, T; MIZUTANI, M. 2018. Generation of α-solanine-free hairy roots of potato by CRISPR/Cas9 mediated genome editing of the St16DOX gene. *Plant Physiology and Biochemistry* 18: 30184-0.

NEKRASOV, V; STASKAWICZ, B; WEIGEL, D; JONES, JDG; KAMOUN, S. 2013. Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease. *Nature Biotechnology* 31: 691-693.

NEKRASOV, V; WANG, C; WIN, J; LANZ, C; WEIGEL, D; KAMOUN, S. 2017. Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. *Scientific Reports*7: 482.

NEVES, CG; AMARAL, DOJ; PAULA, MFB; NASCIMENTO, GC; PASSOS, OS; SANTOS, MA; OLLITRAULT, P; GESTEIRA, AS; LURO, F; MICHELI, F. 2018. Characterization of tropical mandarin collection: implications for breeding related to fruit quality.*Scientia Horticulturae* 239: 289-299.

NONAKA, S; ARAI, C; TAKAYAMA, M; MATSUKURA, C; EZURA, H. 2017. Efficient increase of ɣ-aminobutyric acid (GABA) content in tomato fruits by targeted mutagenesis. *Scientific Reports* 7: 7057.

PARMAR, N; SINGH, KH; SHARMA, D; SINGH, L; KUMAR, P; NANJUNDAN, J; KHAN, YJ; CHAUHAN, DK; THAKUR, AK. 2017. Genetic engineering strategies for biotic and abiotic stress tolerance and quality enhancement in horticultural crops: a comprehensive review. *3 Biotech* 7: 239.

PINGALI, PL; FEDER, G.017. Expanding the biotechnology revolution. In: PINGALI, PL; FEDER, G (eds). *Agriculture and rural development in a globalizing world: challenges and opportunities.* Earthscan, London: Routledge p. 204-205.

PUCHTA, H. 2005. The repair of double-strand breaks in plants: Mechanisms and consequences for genome evolution. *Journal of Experimental Botany* 56: 1-14.

SHAN, Q; WANG, Y; LI, J; ZHANG, Y; CHEN, K; LIANG, Z; ZHANG, K; LIU, J; XI, JJ; QIU, JL; GAO, C. 2013. Targeted genome modification of crop plants using a CRISPR-Cas system. *Nature Biotechnology* 31: 686-688.

SONG, G; JIA, M; CHEN, K; KONG, X; KHATTAK, B; XIE, C; LI, A; MAO, L. 2016. CRISPR/Cas9: A powerful tool for crop genome editing. *The Crop Journal* 4: 75-82.

SOYK, S; MÜLLER, NA; PARK, SJ; SCHMALENBACH, I; JIANG, K; HAYAMA, R; ZHANG, L; VAN ECK, J; JIMÉNEZ-GÓMEZ, JM; LIPPMAN, ZB. 2018. Variation in the flowering gene SELF PRUNING 5G promotes day-neutrality and early yield in tomato. *Nature Genetics* 49: 162-168.

THE POTATO GENOME SEQUENCING CONSORTIUM. 2011. Genome sequence and analysis of the tuber crop potato. *Nature* 475: 189-195.

THE TOMATO GENOME CONSORTIUM. 2012. The tomato genome sequence provides insights into fleshy fruit evolution. *Nature 485 : 635-641.*

TIAN, S; JIANG, L; GAO, Q; ZHANG, J; ZONG, M; ZHANG, H; REN, Y; GUO, S; GONG, G; LIU, F; XU, Y. 2017. Efficient CRISPR/Cas9-based gene knockout in watermelon.*Plant Cell Reports* 36: 399-406.

UETA, R; ABE, C; WATANABE, T; SUGANO, SS; ISHIHARA, R; EZURA, H; OSAKABE, Y; OSAKABE, K; 2017. Rapid breeding of parthenocarpic tomato plants using CRISPR/Cas9. *Scientific Reports* 7: 507.

VAN DEN BROECK, G; MAERTENS, M. 2016. Horticultural exports and food security in developing countries. *Global Food Security* 10: 11-20.

WANG, W; PAN, Q; HE, F; AKHUNOVA, A; CHAO, S; TRICK, H; AKHUNOV, E. 2018. Transgenerational CRISPR/CAS9 activity facilitates multiplex gene editing in allopolyploid wheat. *TheCRISPR Journal* 1: 65-74.

WANG, Y; CHENG, X; SHAN, Q; ZHANG, Y; LIU, J; GAO, C; QIU, JL. 2014. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature Biotechnology* 32: 947-951.

WOLTER, F; PUCHTA, H. 2018.The CRISPR/CAS revolution reaches the RNA world: CAS 13, a new swiss army knife for plant biologist. *The Plant Journal* 94: 767-775.

XU, C; PARK, SJ; VAN ECK, J; LIPPMAN, ZB. 2016. Control of inflorescence architecture in tomato by BTB/POZ transcriptional regulators. *Gene & Development* 30: 2048-2061.

YU, QH; WANG, B; LI, N; TANG, Y; YANG, S; YANG, T; XU, J; GUO, C; YAN, P; WANG, Q; ASMUTOLA, P. 2017. CRISPR/Cas9-induced targeted mutagenesis and gene replacement to generate long-shelf-life tomato lines. *Scientific Reports* 7: 11874.

ZETSCHE, B; GOOTENBERG, JS; ABUDAYYEH, OO; SALYMAKER, IM; MAKAROVA, KS; ESSLETZBICHLER, P; VOLZ, SE; JOUNG, J; OOST, J; REGEV, A; KOONIN, EV; ZHANG, F. 2015. Cp1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Molecular Cell* 163: 759-771.

ZHANG, Y; LIANG, Z; ZONG, Y; WANG, Y; LIU, J; CHEN, K; QIU, JL; GAO, C. 2016. Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. *Nature Communications* 7: 12617.

ZHOU, J; WANG, G; LIU, Z. 2018. Efficient genome-editing of wild strawberry genes, vector development, and validation. *Plant Biotechnology Journal* 12922.

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Where you read: CORTE, LÍGIA ERPEN-DALLA
Read: ERPEN-DALLA CORTE, LÍGIA

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