SUNSHINE AGRICULTURE
e-Newsletter

ISSN (E): 2583 – 0821

Sun. Agri.:e- Newsletter, (2026) *6*(9), 44-49

Article ID: 662

Application of genome editing technique in vegetable crops

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Open Access

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Available online at
www.sunshineagriculture.vitalbiotech.org

Article History
Received: 09.09.2026
Revised: 14.09.2026
Accepted: 19.09.2026

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INTRODUCTION

India grows vegetables on about eleven million hectares and harvests more than two hundred million tonnes a year, a figure second only to China. The distance between that output and what the crop can actually give is caused by wilts, viruses, borers, poor keeping quality as well as a narrow base of cultivated types. A breeder who tries to correct one of these faults by crossing has to carry the entire genome of the donor parent along with the useful gene, then spend six to eight generations pushing out everything that was not wanted.

Genome editing changes that arithmetic. It alters a few bases at a spot chosen in advance inside a variety that farmers already grow, leaving the rest of the genome untouched. The change produced is of the kind mutation breeding has been making for eighty years with gamma rays or ethyl methane sulphonate, except that its position is decided by the breeder instead of being left to chance.

Tools available for genome editing

Three families of tools reached crop research one after another. Zinc finger nucleases came first, followed by transcription activator like effector nucleases. Both cut where they are told to cut, yet each new target needs a fresh protein to be engineered, which is slow costly work. The third tool, the CRISPR associated protein 9 system, was described in 2012 and reached plants within a year. Its address is carried by a short RNA molecule that any laboratory can order cheaply, so moving to a new target means changing twenty bases in the guide rather than rebuilding a protein. Base editors and prime editors have since widened the range further.

Mechanism of editing

The guide RNA carries twenty bases that match the target sequence. Cas9 first searches the genome for a short signal called the protospacer adjacent motif, which for the Cas9 of Streptococcus pyogenes is any base followed by two guanines.

It then unwinds the neighbouring DNA and checks it against the guide. A full match leads to a clean break in both strands about three bases from the motif, as shown in Fig. 1.

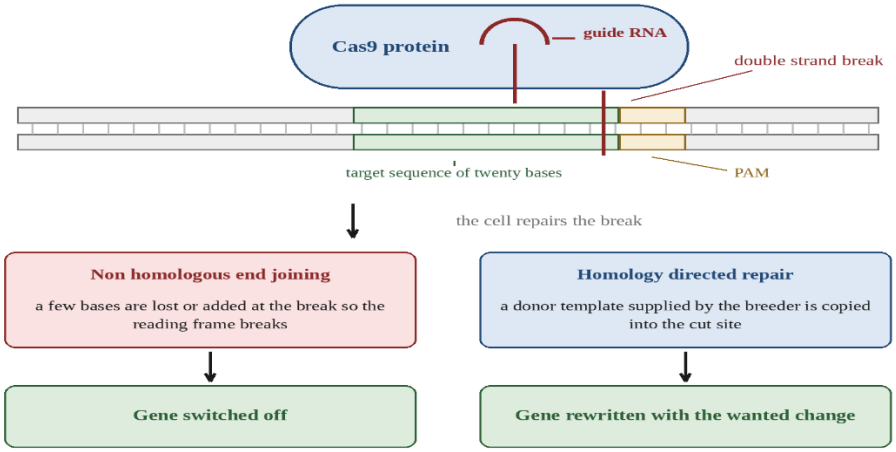


Figure 1: Recognition of the target site by Cas9 and the two routes of repair

Repair of that break is done by the plant itself. Non homologous end joining is quick but careless, so a few bases are gained or lost at the join, the reading frame is disturbed and the protein coded by the gene is lost. This is how a susceptibility gene or a browning enzyme is switched off. Homology directed repair copies a donor template into the cut, yet it is far less frequent in plant cells, which is why most published work in vegetables consists of knockouts.

Procedure followed in a vegetable crop

Work begins with a gene whose function is already known from mutants or expression studies. The guide is designed against the sequence of the genotype to be edited, because a single mismatch between guide and variety can stop the cut. Agrobacterium tumefaciens is the usual carrier of the construct for tomato, potato as well as brinjal, while protoplasts and ribonucleoprotein complexes are preferred where a product free of foreign DNA is wanted from the beginning.

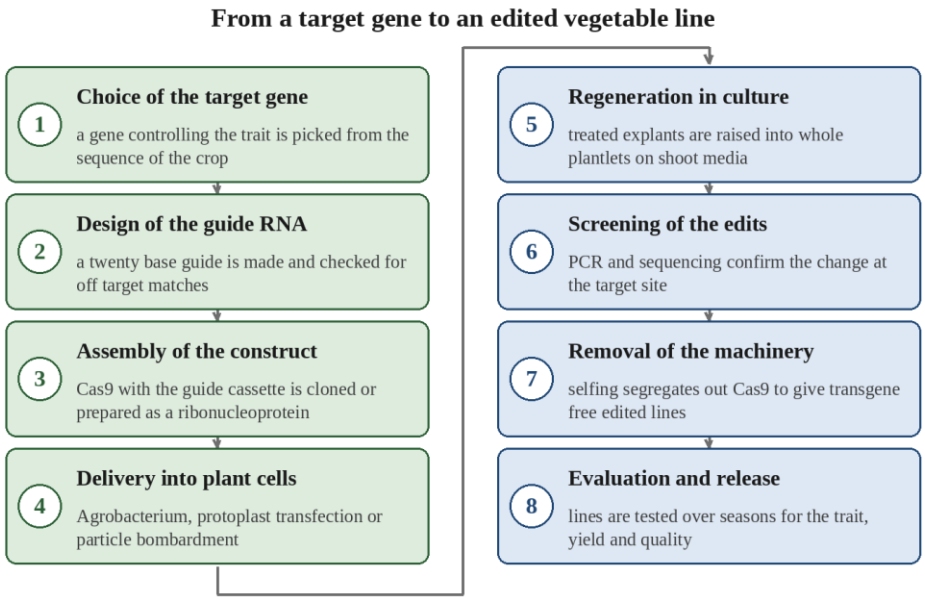


Figure 2: Sequence of steps followed in an editing programme

Regeneration is the real bottleneck. Tomato and potato throw shoots readily from cotyledon or internode explants, whereas many cucurbits and cole types regenerate so poorly that few edited plants come out of a large experiment. Plants with confirmed edits are then selfed so that the region carrying Cas9 segregates out, leaving a defined mutation with no foreign DNA.

Genome editing in different vegetable crops

Tomato holds the largest share of the published work, yet every major vegetable group now has at least one clear success to show. The examples below give the gene that was cut in each crop along with the change that followed.

Tomato: Knocking out the SlMlo1 gene of the cultivar Moneymaker produced Tomelo, a powdery mildew resistant line free of any transgene. A cut in the RIN locus slowed the softening of ripe fruit while editing of SlIAA9 gave seedless fruit that sets without pollination. Cuts placed in the promoter of CLV3 raised a series of plants running from normal fruit to very large fruit with extra locules.

Potato: All four copies of a gene have to be hit in this tetraploid crop, which editing manages in a single cell. Removal of granule bound starch synthase yields amylose free waxy starch. Knockout of the vacuolar invertase gene stops reducing sugars from building up in cold store, so chips fry lighter with much less acrylamide, while switching off a polyphenol oxidase gene keeps cut tubers pale.

Cucumber: The host factor eIF4E was knocked out. Potyviruses cannot translate their own genome without this protein, so one edit gave resistance to cucumber vein yellowing virus, zucchini yellow mosaic virus as well as papaya ring spot virus together.

Watermelon: The method was first standardised on the phytoene desaturase gene, which bleaches the seedling and shows at a glance how well the cut worked. Base editing of acetolactate synthase then gave plants tolerant of sulfonylurea herbicide.

Cabbage and cauliflower: Heritable mutations were obtained in Brassica oleracea by knocking out a gibberellin biosynthesis gene, which gave dwarf plants. Present work is aimed at head compactness, delayed bolting as well as clubroot resistance.

Brinjal: Three polyphenol oxidase genes were cut together so the flesh stays pale much longer after slicing, which answers the discolouration that pulls down the price of cut produce.

Chilli: Knockout of the ethylene responsive factor CaERF28 gave resistance to anthracnose, the disease that ruins ripe fruit across the humid tracts of eastern India.

Carrot: The flavanone three hydroxylase gene was edited to change the anthocyanin content of the root, which shifts root colour.

Other crops: Work in onion, okra, pea as well as the leafy vegetables is still at the stage of standardising transformation and regeneration, since these crops give back few plants from culture. Table 1 lists the main achievements reported so far.

Improvement of quality and nutritive value

Editing has worked well on compounds the plant already makes. Multiplex editing of five carotenoid pathway genes raised lycopene in ripe tomato several fold. Truncation of the SlGAD3 gene let gamma aminobutyric acid accumulate in the fruit, and a tomato built on that work went on sale in Japan in 2021 as the first edited food crop offered directly to consumers. Fig. 3 brings together the traits changed so far in the main crops.

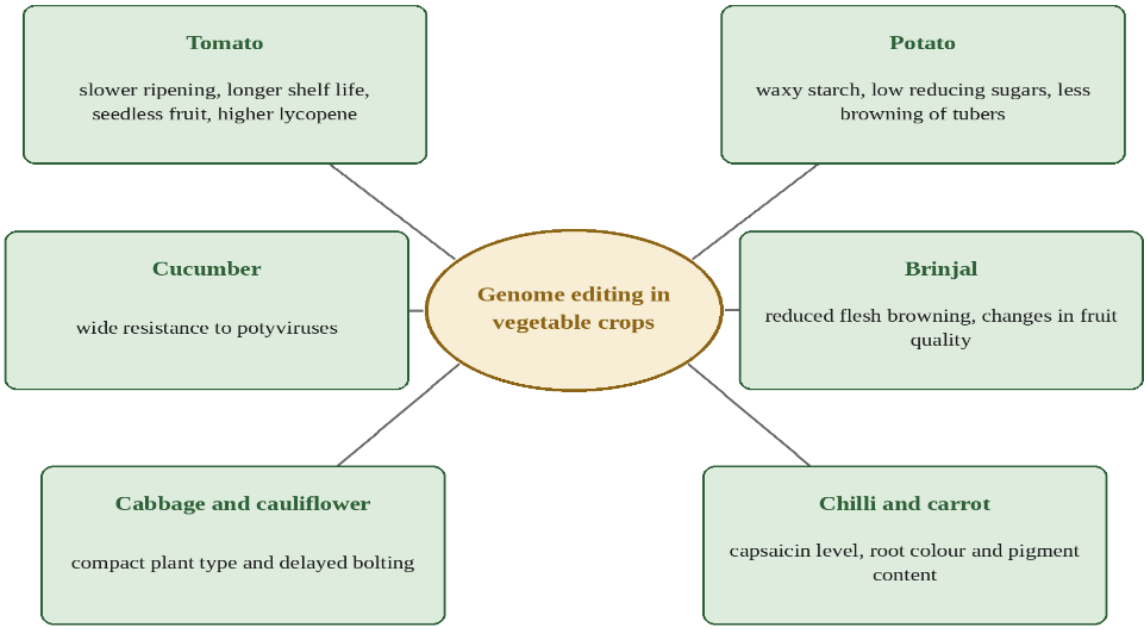


Figure 3: Traits altered by genome editing in the major vegetable crops

Table 1: Genome editing work reported in the main vegetable crops

Crop	Gene edited	Tool used	Change obtained
Tomato	SlMlo1	CRISPR/Cas9	Resistance to powdery mildew
Tomato	RIN	CRISPR/Cas9	Slow softening of ripe fruit
Tomato	SlIAA9	CRISPR/Cas9	Seedless fruit without pollination
Tomato	SlGAD3	CRISPR/Cas9	Higher gamma aminobutyric acid
Tomato	Five carotenoid genes	Multiplex CRISPR/Cas9	Several fold rise in lycopene
Potato	GBSS	Cas9 RNP	Amylose free waxy starch
Potato	VInv	TALEN	Low reducing sugars in cold store
Potato	StPPO2	Cas9 RNP	Less browning of cut tubers
Cucumber	eIF4E	CRISPR/Cas9	Resistance to three potyviruses
Watermelon	ClALS	Base editing	Tolerance to sulfonylurea herbicide
Cabbage	BolC.GA4.a	CRISPR/Cas9	Dwarf plant type
Brinjal	SmelPPO4, 5 and 6	CRISPR/Cas9	Pale flesh after cutting
Chilli	CaERF28	CRISPR/Cas9	Resistance to anthracnose
Carrot	DcF3H	CRISPR/Cas9	Altered anthocyanin in root

Domestication of wild relatives

Solanum pimpinellifolium is a wild tomato that tolerates salinity and disease though it bears very small fruit. Six loci governing plant type, fruit size, fruit number as well as vitamin content were edited in it, and within one generation the result was a plant that looked domesticated while keeping the toughness of the wild parent. The same was done in ground cherry. The approach turns breeding around, since the wild species itself is domesticated instead of a wild gene being dragged into a cultivated background over many years.

Regulatory status in India

The Ministry of Environment, Forest and Climate Change exempted genome edited plants of the SDN 1 and SDN 2 categories

from the rules that govern genetically modified organisms in 2022, provided no foreign DNA remains in the final product. The Department of Biotechnology issued safety assessment guidelines in the same year. Two genome edited rice lines were announced by the Indian Council of Agricultural Research in 2025 under this framework, a precedent that vegetable programmes in the country are now following.

Constraints and future scope

Three problems still hold the technology back in vegetables. Regeneration protocols remain poor for several crops, so the reagents often go into a genotype that grows well in culture rather than into the variety the farmer sows. Off target cuts have to be ruled out by sequencing before a line is advanced. Most useful patents sit with a few institutions abroad, which raises the cost of reaching the market. None of these is a barrier of principle. Genotype independent regeneration using developmental regulators such as WUSCHEL and BABY BOOM already works in several species. The realistic prospect for the coming decade is a familiar variety, sown by the same farmer, that keeps two weeks longer on the shelf or shrugs off a virus that used to take a third of the crop.

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