Genome editing: a boon for plant biologists, breeders and farmers

K. C. Bansal, Kutubuddin A. Molla and Viswanathan Chinnusamy

Science-led technologies have greatly benefitted humankind in various fields. India attained food security through Green Revolution in the mid-1960s–70s, which was a result of developing new high-yielding varieties of wheat and rice introgressed with semi-dwarfing genes through conventional plant breeding methods. Consequently, food grain production increased substantially in the country. Conventional plant breeding, which is entirely based on phenotypic selection, with limited knowledge of the associated gene(s) with the selected trait(s), is time-consuming and takes about 10–12 years to develop a new crop variety. Years of extensive research using DNA-based molecular tools helped breeders to hasten the process of breeding by selection through molecular markers and shuttle breeding. Genetic variation is the key prerequisite for crop improvement. Since the accumulation of genetic variation due to spontaneous mutation is a very long process, scientists learned to rapidly induce variation in DNA using physical or chemical mutagens. Mutation breeding generates random variation in the genome and has led to the release of more than 3200 plant varieties in 70 countries (IAEA database). However, obtaining desirable mutants through induced mutagenesis is time and labour-intensive. Further, to add new traits of economic importance, scientists discovered the methods of transferring genes from unrelated sources to plants, and subsequently, GM crop varieties were developed with several useful traits through the process of genetic engineering. In recent times, an unprecedented revolution has occurred due to the development of the novel technology called genome editing, which has emerged as a new breeding tool with enormous potential. Genome editing allows modifications in an organism's native DNA at a pre-determined genomic locus in a precise and targeted manner.

Genome editing typically involves targeted cleavage of the plant's double-stranded DNA by the CRISPR-associated (Cas) endonuclease protein guided by a customizable small RNA. The Cas-induced double-strand break (DSB) is repaired by the cell's repair machinery. Plants predominantly use the imprecise non-homologous end-joining (NHEJ) pathway, which introduces small deletion/addition of nucleotides, often disrupting (knock-out) the gene function. Similarly, NHEJ can also mediate the alteration of *cis*-regulatory DNA elements in the promoters for altering gene expression. The most profound impact of genome editing on crop breeding is the removal of deleterious genes. The technology also allows precise substitution of nucleotides and insertion (knock-in) of DNA sequences at a predefined position using homologous template sequences through a homology-directed repair (HDR) system. This way, the genome editing technologies (SDN1 and SDN2) allow modification of plants' genetic elements and are not dependent on foreign DNA/gene insertion for incorporating a particular trait.

Classical genetic engineering, on the other hand, causes random integration of the transgene in the genome. Therefore, GM crops need to go through a lengthy developmental and clearance process through a stringent regulatory regime. Interestingly, many countries treat genome-edited crops as non-GMOs because of the absence of foreign DNA in the final product, i.e. improved crop variety, is foreign DNA-free.

Recognizing the importance of this powerful technology for Indian agriculture, the Government of India has exempted the SDN1 and SDN2 categories of genome-edited crops from biosafety assessment under the Rule 20 of Rules 1989 of the Environment (Protection) Act, 1986 (Ref. C-12013/3/2020-CS-III, dated March 30, 2022). This is a real boon to Indian plant biologists and breeders to use genome editing tools for basic research in functional genomics and gene discovery as well as translational research for developing high-yielding crop varieties and hybrids to meet the challenges posed by climate change, shrinking natural resources and malnutrition. Genome editing could generate new varieties, which require fewer inputs such as agrochemicals, water and fertilizers, and produce more output enabling the increase in farmers' income.

The technology is beset with great potential for next agricultural revolution, specifically by developing crops with desired traits for sustainable crop production. Genome editing has successfully generated crops with improved novel traits that were technically difficult through traditional breeding or genetic engineering. For instance, the wheat plant has been made resistant to the powdery mildew fungal pathogen through *Mlo* gene editing[1]. Editing a single gene in rice increased yield and tolerance to salinity and drought stresses[2]. Another classic example is the generation of genome-edited rice with broad-spectrum resistance to bacterial blight pathogen by mutating the promoter of sucrose transporter genes[3]. Numerous novel promoter allelic combinations were generated in tomato plants through *cis*-elements editing to improve fruit size, plant architecture and inflorescence branching[4]. Recently, tomato plants were edited to produce a precursor to vitamin D[5]. Remarkably, gene-edited crops like high-GABA tomato (in Japan) and high oleic acid soybean (in the USA) and gene-edited fishes for higher productivity and feed efficiency (in Japan) have reached the market within a record time.

Notably, the genetic alterations mentioned above were achieved by changing only a few bases in a targeted manner, compared to random mutagenesis attempted in the past through chemical or physical mutagens. As a result, the genome-edited crops developed with minor modifications in their native genes remain indistinguishable from traditionally bred varieties that could have been developed by any conventional breeding methods, including induced mutations. Several complex agronomic traits need immediate attention, and now we can use CRISPR-based multiplex genome editing to improve complex traits with relative ease.

More importantly, genome editing will allow enhanced use of crop diversity by mimicking useful genetic variation present in landraces and wild germplasm collections in the Indian National Gene Bank, directly in elite cultivars. Crop wild relatives with desired traits like disease resistance and abiotic stress tolerance are now amenable for rapid *de novo* domestication using genome editing. For example, wild tomato[6,7], wild rice[8] and orphan crops like ground cherry[9] were edited for accelerated *de novo* domestication.

It has been a long-sought plant breeding goal to clonally propagate hybrid seeds. Genome editing technology has made it possible to generate hybrid rice and maize that can be clonally produced through parthenogenesis or haploid induction[10,11], thus

Table 1. Priority crops and traits for genetic improvement through genome editing for Indian agriculture

Category	Crop	Prioritized traits	Potential candidate gene(s)*	SDN1, SDN2 or other remarks
Cereal	Rice	High grain yield	*Gn1a*, *GS3*, *GW2*, *DEP1*, *TB1*, *IPA1* (promoter edits), *PYL*1/4/6	SDN1
		Resistance to diseases: bacterial leaf blight (BLB), blast, sheath blight	BLB: *SWEET11/13/14* Blast: *ERF922*, *BSRK1*	SDN1
		Resistance to insect-pests: brown plant hopper	*CYP71A1*	SDN1
		Herbicide tolerance for direct-seeded rice	*ALS*, *ACCase*, *EPSPS*	Base/Prime editing (SDN1); SDN2
		Enhanced water (WUE) and nitrogen use efficiency (NUE)	WUE: *EPF-LIKE9*, *or STOMAGEN*, *EPFL10* NUE: *NRT1.1B*	Base/prime editing (SDN1)
		Tolerance to drought and salinity	*DST, RR22*	SDN1
		High Fe and Zn content in grains	*GW2*, *HRZ* (Hemerythrin RING Zinc finger)	SDN1
	Wheat	Heat tolerance	MULTIPROTEIN BRIDGING FACTOR 1 (*MBF1*)	SDN1 – Promoter editing for enhancing the expression; more genes to be identified
		Resistance to lodging	Not known yet**	
		Hectolitre weight	Not known yet**	
		Grain yield	CYTOKININ RESPONSE1 REPRESSOR1 (*ARE1*)	SDN1
			CKX	SDN1
			SPL	SDN1 in promoter; SDN2
		High nitrogen use efficiency	CYTOKININ RESPONSE1 REPRESSOR1 (*ARE1*)	SDN1
		High Zn and Fe content	*HRZ*	SDN1
Oilseeds	Mustard	Plant type: increased secondary branching	*MAX1*, *TT2*, *TT8*	SDN1
		Sclerotinia (stem rot) resistance	*Ferulate-5-hydroxylase*	SDN1
		Resistance to Orobanche	*ALS*	SDN1 (base/prime editing); SDN2
		Resistance to *Alternaria* leaf spot	Not known yet**	
		Oil quality: low glucosinolate and low erucic acid	Low Glucosinolate: *MYB28*; *GTR* Low erucic acid: *Fatty acid elongase1 (FAE1)*	SDN1
	Ground-nut	Bushy plant type	Not known yet**	
		Resistance to diseases: leaf spot, rust and stem rot	Not known yet**	
		Drought tolerance	Not known yet**	
		High oleic acid in seeds	*FAD2*	SDN1
	Soybean	Photo-insensitivity (Early flowering/early maturity)	*E1*, *E2* and *E3* genes	SDN1
		Herbicide tolerance	*ALS*, *EPSPS*	Base/prime editing (SDN1); SDN2
		High oleic acid in seeds	*FAD2-1A* and *FAD2-1B* genes	SDN1
Pulses	Chickpea	Resistance to diseases: dry rot, *Botrytis* grey mould, Ascochyta Blight	Not known yet**	
		Pod borer resistance	Not known yet**	
		Tolerance to drought and salt stress	Not known yet**	
		Herbicide tolerance	*ALS*, *EPSPS*	Base/prime editing (SDN1); SDN2
	Pigeon pea	Synchronized maturity/early maturity	Not known yet**	
		Photo- and thermo-insensitivity	Not known yet**	
		Drought tolerance	Not known yet**	
		Herbicide tolerance	*ALS*, *EPSPS*	Base/prime editing (SDN1); SDN2
		Resistance to diseases: *Alternaria* blight, anthracnose	Not known yet**	
		Pod borer resistance	Not known yet**	
	Green gram	Resistance to yellow mosaic virus	Not known yet**	
		Resistance to powdery mildew	Not known yet**	*MLO* orthologous gene may work
		Drought and heat tolerance	Not known yet**	

(*Contd*)

Table 1. (*Contd*)

Category	Crop	Prioritized traits	Potential candidate gene(s)*	SDN1, SDN2 or other remarks
Cash crops	Sugarcane	High yield	Not known yet**	Since sugarcane has a high level of polyploidy, dominant mutations that govern traits can be selected, as even if a few homeologs are edited, it will produce desirable traits. For example, herbicide resistance by editing *ALS*
		Resistance to red rot disease	Not known yet**	
		Juice quality (>18% sucrose in a 10-month-old crop)	Not known yet**	
	Cotton	High yield: increased boll weight and number	Not known yet**	
		Increased fibre length and strength	Not known yet**	
		High ginning out-turn (~40%)	Not known yet**	
		Resistance to pink bollworm	Not known yet**	
Fruit and vegetable crops	Banana	Resistance to *Fusarium* wilt Race 1 and TR4 (Panama wilt)	Strong promoter knock-in upstream to *RGA2*	SDN3 SDN1; Tiling deletion screen to identify deletions in the repressor binding *cis*-elements may be attempted to enhance the expression (similar to the promoter deletion work done in *OsIPA1* to enhance expression[16])
		Resistance to Sigatoka leaf spot	Not known yet**	
		Nutritional fruit quality: high beta-carotene and iron	Beta Carotene: *Lycopene epsilon-cyclase*	SDN1
		BBTV (Banana bunchy Top Virus) and BSV (Banana Streak virus)	*eIF4* ORF1/2/3 of integrated endogenous BSV	SDN1
	Tomato	Improved processing quality: total soluble solids (>5%) and acidity (>0.5%)	*Invertase inhibitor (INVINH)*	SDN1
		Male sterility for hybrid seed production	*Ms10(35)*, *GSTAA, LeRBOH*, and *LeRBOHE*	SDN1
		Resistance to tomato leaf curl virus (TLCV) disease	*SlPelo*	SDN1
	Potato	Reduce cold-induced sweetening and acrylamide content	Sweetening: *Vacuolar Invertase* Acrylamide: *Asparagine synthetase 1*	SDN1
		Resistance to diseases: potato virus Y (PVY) and potato New Delhi apical leaf curl virus, bacterial wilt, late blight	PVY: *P3*, *CI*, *Nib*, *CP* viral genes; *eIF4E* and *coilin* host genes Late Blight: *StDMR6-1* and *StCHL1*	SDN1
		Tuber starch quality	*GBSS*, *SBE1* and *SBE2*	SDN1

*Other alternative genes may also work equally well or better than the genes mentioned here.
**Target genes need to be identified and validated on priority for specific crops for these traits through functional genomics approaches. CRISPR-Cas tools can also play a significant role in functional genomics.
The crops listed are prioritized and only indicative.

permitting farmers to save seeds for successive sowings without losing hybrid vigour.

What crops and traits India should focus on?

The availability of efficient genome engineering tools for precise editing would greatly help Indian agriculture by rapidly and accurately creating useful alleles directly in the elite crop varieties. While the green revolution resulted in self-sufficiency with regard to food production that enabled the Government to enact the Right to Food Act-2013 to feed millions of people, now is the time of greater concern to address the issues of climate adaptation of crops, producing more from the shrinking natural resources, increasing input use-efficiency and combating malnutrition.

According to the recent IPCC sixth assessment report (2022), India is one of the most economically stressed countries due to climate change. Major emphasis is, therefore, needed for developing climate-ready crops combining field stress tolerance/resistance to abiotic and biotic stress factors with yield enhancement traits like improved photosynthesis, enhanced acquisition and use efficiency of water, nutrients and other natural resources to produce more with less and fewer inputs (Table 1). The CRISPR-Cas technology is particularly useful in developing these traits through multiplex gene editing by creating and combining allelic diversity from widely adapted diverse germplasm resources like crop wild relatives and landraces into elite crop genotypes.

Efforts are already in progress in India to develop improved genome-edited varieties in several crops by both public and private sector laboratories, however, mostly in rice as an important food security crop. Dwindling freshwater resources, rising day and night temperatures, and increasing soil salinity pose major challenges for rice cultivation in India. Hence, the target traits for genetic improvement of rice include water use efficiency, nitrogen use efficiency, abiotic stress tolerance, grain quality and productivity (Table 1).

It is desired that emphasis will be given more to pulses and oilseed crops for raising productivity levels, improving nutritional quality, and developing new varieties resistant to heat and drought stresses, pests, and pathogens, to help reduce India's dependency on imports. However, the unavailability of functionally characterized genes for these traits limits the application of genome editing. Hence, besides focusing on genome editing for product development, genome editing for gene discovery should be an important thrust area in these and other prioritized crops (Table 1).

Weeds pose a major threat to modern agriculture. Herbicide-mediated weed management minimizes greenhouse gas emissions from soil. Advanced genome editing technologies are in use to develop herbicide-tolerant crop varieties[12]. India needs to use the power of this technology to develop crop varieties tolerant to different herbicides, aimed at direct-seeded rice.

Latest scientific developments and advances in genome editing

Unparalleled advancement in genome editing technologies has produced powerful tools that will propel plant breeding to a new height with utmost precision. CRISPR-Cas gave rise to several game-changing advanced breeding tools beyond the limited applicability of knocking-out genes. Most prominent is the base editing technology that allows us to modify nuclear and organellar genomes at a single base resolution[12]. Precise alteration of one target nucleotide into another is done with available base editors, CBE (for C to T), ABE (for A to G) and CGBE (for C to G). Base editors can be used for imparting herbicide tolerance, disease tolerance, yield enhancement, altering the nutrient composition and many more traits[12]. Prime editing, another precise editing technique, has broader implications as it could be used to generate any point mutations and introduce predefined small insertions and deletions at target loci[13]. Although the efficiency of prime editing is much lower compared to base editing and canonical CRISPR-Cas NHEJ mutation[12], it would prove to be a boon for crop improvement once the efficiency is increased by further development and refinement.

Major bottlenecks in using genome editing technologies are the non-availability of characterized genes, poor reagent delivery system and unavailability of regeneration protocol for many elite crop genotypes. Recent reports showed that expression of developmental regulators *WUSCHEL* and *BABYBOOM* promote organogenesis and could be used in breaking recalcitrancy[14]. Recently, Yiping Qi and colleagues devised CRISPR-COMBO to overcome the problem of regeneration during genome editing[15]. They combined the power of CRISPR-gene activation and targeted mutagenesis/base editing. Simultaneous activation of *BABYBOOM* or *WUSCHEL* significantly increased the efficiency of targeted mutagenesis or base editing in rice and poplar while promoting plant regeneration. CRISPR-COMBO offers great potential to overcome the regeneration barrier to performing genome editing in diverse crop genotypes in India.

Future outlook

To harness the full potential of genome editing technologies, urgent attention is needed to simplify the process of generating genome-edited crop events by developing efficient genetic transformation systems in elite crop cultivars. Since vegetatively propagated crops cannot be made foreign DNA-free through sexual segregation, priority should be on establishing DNA-free gene editing protocols in such crops to use the new guidelines from Government of India. Transient expression of genome editing reagents, for example, ribonucleoprotein (RNP) complex, is an attractive way to achieve targeted modification without any remnant of the foreign DNA/reagents and is applicable to both sexually and asexually propagated plants. Similarly, successful genetic transformation of wild crop species and innovations in developing simplified methods for introducing genetic material will prove useful for accelerating crop neo-domestication with improved traits related to climate resilience. To accelerate the pace of applying this powerful technology for plant breeding innovations, India needs to equally focus on funding projects on gene discovery and transformation system establishment of all important major and minor crops. With time, these technologies could pave the way for introducing biological nitrogen fixation in non-leguminous species, including cereals and oilseed crops.

Investment is also needed in human resource development for young biologists and plant breeders, and capacity building across institutions to derive full benefits of this novel technology in all sectors of agriculture. Original research on developing novel genome editing tools by exploring the diversity of Cas effectors from the naturally available microbes would reduce the dependency of India on other countries. A congenial environment for allowing Indian scientists to collaborate with experts from other countries would also play a significant role in advancing genome editing research and product development in India.

1. Li, S. *et al*., *Nature*, 2022, **602**, 455–460.
2. Kumar, S. *et al*., *Physiol. Mol. Biol. Plants*, 2020, **26**, 1099–1110.
3. Oliva, R. *et al*., *Nat. Biotechnol.*, 2019, **37**, 1344–1350.
4. Rodríguez-Leal, D., Lemmon, Z. H., Man, J., Bartlett, M. E. and Lippman, Z. B., *Cell*, 2017, **171**, 470–480.
5. Li, J. *et al*., *Nat. Plants*, 2022, **8**, 611–616.
6. Zsögön, A. *et al*., *Nat. Biotechnol.*, 2018, **36**, 1211–1216.
7. Li, T. *et al*., *Nat. Biotechnol.*, 2018, **36**, 1160–1163.
8. Yu, H. *et al.*, *Cell*, 2021, **184**, 1156–1170.
9. Lemmon, Z. H. *et al*., *Nat. Plants*, 2018, **4**, 766–770.
10. Khanday, I., Skinner, D., Yang, B., Mercier, R. and Sundaresan, V., *Nature*, 2019, **565**, 91–95.
11. Wang, C. *et al*., *Nat. Biotechnol.*, 2019, **37**, 283–286.
12. Molla, K. A., Sretenovic, S., Bansal, K. C. and Qi, Y., *Nat. Plants*, 2021, **7**, 1166–1187.
13. Anzalone, A. V. *et al*., *Nature*, 2019, **576**, 149–157.
14. Lowe, K. *et al*., *Plant Cell*, 2016, **28**, 1998–2015.
15. Pan, C. *et al*., *Nat. Plants*, 2022, **8**, 513–525.
16. Song, X. *et al.*, *Nat. Biotechnol.*, 2022; https://doi.org/10.1038/s41587-022-01281-7

ACKNOWLEDGEMENTS. We thank respective crop breeders for helping us in identifying the crop-wise priority traits: Ashok K. Singh and Gopala Krishnan (rice), Rajbir Yadav (wheat), D. K. Yadava (mustard), Sanjeev Gupta (soybean, groundnut, chickpea, pigeon pea, green gram), Nita Khandekar, Sanjay Gupta, Giriraj Kumawat (soybean), Bakshi Ram (sugarcane), V. N. Waghmare (cotton), S. Uma (banana), T. K. Behera (tomato) and Salej Sood (potato). We also thank Dr Yiping Qi, University of Maryland, USA for his valuable comments on the manuscript. Work in VC's Lab is funded by NASF, ICAR and DBT, Government of India.

K. C. Bansal is in the National Academy of Agricultural Sciences, New Delhi 110 012, India; Kutubuddin A. Molla is in the ICAR-National Rice Research Institute, Cuttack 753 006, India; Viswanathan Chinnusamy is in the ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India.*
**e-mail: kcbansal27@gmail.com*