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National Academy of Agricultural Sciences

Regulatory Framework for Genome Edited Plants: Accelerating the Pace and Precision of Plant Breeding

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Preface

The agricultural sector in India has made unparalleled progress in increasing the production and productivity of different farm commodities including food grains, vegetables, fruits, flowers, fibres, fisheries, dairy and poultry since independence. Improved plant varieties and animal breeds, developed through continuously evolving breeding tools have played a key role in this in addition to optimizing natural resources utilization for realizing the true potential of these varieties.

Conventional breeding involves selection of superior types from the existing natural variation in the germplasm arising through spontaneous mutation and recombination or those produced by chemical or radiation induced mutation and artificial hybridisation. However, towards the end of 20th century, the new recombinant DNA or GM technology has revolutionised the plant breeding methodology by enabling transfer of genes across the species. Typified by the success of Bt-cotton in India, it has also brought an added responsibility to ensure the bio-safety of GMO products through a stringent regulatory framework. Genome editing has emerged as the latest and most precise breeding tool available that allows creation of directed precise mutations in the genome without addition of any foreign DNA, which makes the products indistinguishable from the natural mutations. The world is rapidly adopting genome editing technology which is now a billion dollar industry. Therefore, it is imperative to have an appropriate policy and regulatory framework for the genome edited organisms.

This Policy Brief developed by the National Academy of Agricultural Sciences outlines not only the expected benefits of powerful genome editing technology to Indian Agriculture, but also the global and national status of the regulatory framework for the commercialisation of genome edited plants and recommendations for devising an efficient regulation for genome edited varieties of food grains, horticultural and agroforestry plants.

The Academy can, therefore, play a catalytic role in streamlining the regulation of genome-edited plants as the products developed through SDN1 and some SDN2 approaches are indistinguishable from the products of conventional breeding. Hence, these products should be made available to the farmers in the shortest time possible.

On behalf of the Academy, I am thankful to the guidance of Dr R. S. Paroda, Prof R. B. Singh, Prof Deepak Pental, Prof B. D. Singh and others who significantly contributed. I compliment the Convener, Prof N. K. Singh, Core Committee members and all the discussants for their valuable efforts in developing the policy brief, as well as Dr Kusumakar Sharma and Dr P. S. Birthal for their editorial help.

Dated : 31 July, 2020

Trilochan Mohapatra
President
National Academy of Agricultural Sciences

NATIONAL ACADEMY OF AGRICULTURAL SCIENCES

Round Table Discussion: Genome Edited Organisms

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Discussants

1. Prof Deepak Pental, FNAAS, Ex. VC Delhi Universtiy, New Delhi
2. Dr TR Sharma, DDG (CS), ICAR, New Delhi
3. Dr Anand K. Singh, DDG (Hort) ICAR, New Delhi
4. Dr Shelly Praveen, Head, Biochemistry, IARI, New Delhi
5. Dr Y.J. Parashuram, Regulatory Manager, J.K. Agri Genetics Ltd,
6. Dr Gurinder Jeet Randhawa, NBPGR, New Delhi
7. Dr M.K. Reddy, Group Leader (Crop Improvement Group), ICGEB, New Delhi
8. Dr Rohini Sreevathsa, Member Secretary IBSC, ICAR-NIPB New Delhi
9. Dr Kuldeep Singh, Director NBPGR, New Delhi
10. Dr Archana Sachdev, Principal Scientist, Division of Biochemistry, IARI, New Delhi
11. Dr C. Vishwanathan, Head, Div. of Plant Physiology, IARI, New Delhi,
12. Prof D. Pattanayak, Professor ICAR-NIPB, New Delhi
13. Dr Yogesh Vikal, Senior Geneticist, PAU, Ludhiana
14. Dr Shivendra Bajaj, Federation of Seed Industry of India, New Delhi
15. Dr Deepak Singh Bisht, Scientist, ICAR-NIPB, New Delhi
16. Dr C. Bharadwaj, Principal Scientist, Division of Genetics, IARI, New Delhi
17. Dr S.R. Bhat, Former Principal Scientist, ICAR-NIPB and former member RCGM
18. Dr Sanjeev Kalia, Regulatory Affairs Manager, BASF India Limited, New Delhi
19. Dr Jagdeep Singh Sandhu, Biotechnologist, PAU, Ludhiana
20. Shri Jagdish Singh, Progressive farmer, Sri Ganaganagar, Rajasthan
21. Shri Wazir Singh, Progressive farmer, The Uchana, Distt. Jind, Haryana
22. Shri Chamkour Singh, Progressive farmer, Jothpur, Pakhar, Distt. Bathinda, Punjab
23. Shri Jagdish Prasad, Progressive Farmer, Hisar
24. Dr Sangeeta D. Mendiratta, Senior Manager, Bayer, New Delhi
25. Dr Anil K. Singh, Secretary NAAS, New Delhi

Regulatory Framework for Genome Edited Plants: Accelerating the Pace and Precision of Plant Breeding

1. Improved Varieties are Crucial to Sustainable Crop Production

Genetic restructuring of major food crop and horticultural plants by infusion of **technological innovations** in **plant breeding** has played a key role in creating and sustaining the Indian and global food security. Indian plant scientists and breeders have developed improved varieties of almost all crops with emphasis on staple food, fibre and horticultural crops like cereals, pulses, oilseeds, sugarcane, cotton, jute, fruits and vegetables over the last century with significant incremental gains in productivity (Singh *et al.*, 2016). A tenfold increase in food grain production during the last hundred years would not have been possible without the genetic improvement of crops complemented with the advances in natural resource management. The earliest breeding approach practiced by **ancient farmers** and **plant breeders** is selection of superior plant types from the **natural variation** produced by **spontaneous mutations** creating novel alleles of the genes and **recombination** of alleles of different genes in the genome. However, plant breeding methods have evolved continuously with innovations in science and technology.

Discovery of sex in plants by Camerarius (1694) and production of first **artificial hybrids** in plants by Fairchild (1716) enlarged the scope of selection from variation created by **artificial hybridization** or **crossing** as a new plant breeding tool. Joseph Koelreuter (J Heredity, 1930) made many artificial crosses in tobacco during 1760-1766 and described the phenomenon of **hybrid vigour** in F_1 hybrids, while Thomas Knight (1759-1835) developed many varieties of apple, pear, peach and grape by artificial hybridisation (Gilman *et al.*, 1905). Analysis of the progenies of artificial F_1 hybrids in garden pea enabled Mendel (1865) to decipher the fundamental **laws of inheritance,** thus laying the foundation of Genetics. Artificial hybridisation became a widely used tool for plant varietal improvement by the end of nineteenth century, leading to establishment of Garton's Agricultural Plant Breeders in England as a commercial concern for cereals and vegetables in 1889. William Farrer revolutionised wheat breeding in Australia by releasing rust resistant variety 'Federation" in 1903 after 20 years of breeding based on Mendelian principles. Breeding by crossing received further boost after rediscovery of Mendel's laws in 1900. In India, selection after crossing led to development of high grain quality disease resistant wheat varieties during 1020s (Mehta and Pal, 1940). Similarly, Ramiah and Ramaswamy (1936) developed semi-dwarf rice varieties with multiple resistance to blast, bacterial leaf blight and brown plant hopper. Rice varieties tolerant to multiple abiotic stresses such as drought, flood and salinity were also developed quite early through artificial hybridisation (Richharia, 1960). This early adoption of artificial hybridization in India was a forerunner to the **Green Revolution** of the 1960s, which was due to breeding of semi-dwarf, photo-thermo-insensitive, input-responsive varieties of wheat and rice for Indian farmers based on **global technological leads** (Swaminathan, 1968). In just four years between 1964 to 1968, Indian plant breeders deployed these dwarfing genes to add productivity gains equal to that attained during the preceding 4000 years. The green revolution was achieved by a "symphony" of science, technology, policies and ready adoption of technology by the Indian farmers.

The journey of Green Revolution has continued unabated with the aim of sustainable productivity enhancement by using **technological interventions** for minimizing negative ecological impacts for which the term **"Evergreen Revolution"** was coined (Swaminathan, 2006). The gains of Green Revolution have been protected, consolidated and further enhanced by identifying and mobilising **new genes** for resistance to different diseases and pests for saving and fortifying the harvest (Nagarajan *et al*., 1978; Reddy and Rao, 1979). **Gene pyramiding** for multiple resistance against biotic stresses has been used as an effective strategy to prevent yield losses in wheat (Cherukuri *et al*., 2005; Gupta *et al*., 2006). The threat of dreaded wheat diseases UG99 and wheat blast have been averted by anticipatory breeding and gene deployment strategies (Joshi *et al*., 2008; Singh *et al*., 2019). Using cross breeding tools, Indian rice scientists boosted the yield potential of Basmati rice fourfold and reduced the crop duration by 20 days without loss of grain quality, making Pusa Basmati 1 and Pusa Basmati 1121 most profitable rice varieties. It has greatly boosted the export earnings from Basmati rice in India from less than 0.5 billion dollars in 2003-04 to 4.5 billion dollars in 2013-14 (Singh *et al*., 2018). The sustained productivity growth has led to transformation of India from a ship-to-mouth economy in 1960s to a food secure nation with the **Right to Food Act 2012** based on home grown foods.

Until 1920s plant breeding depended entirely on natural genetic variation produced by spontaneous mutation, but creation of first artificial or **induced mutations** using X-rays by Stadler (1930) laid the foundation of a new plant breeding method called **mutation breeding**. Soon other forms of ionizing radiation such as gamma rays and neutrons and chemical mutagens such as ethyl methane sulphonate (EMS) were widely used for inducing novel mutations. While physical mutagens were too harsh due to high energy creating double strand breakage and large deletions of DNA, chemical mutagens proved better for inducing useful **point mutations**. One of the earliest known examples of mutation breeding in India is the production of dwarf mutants of rice variety GEB 24 by X-ray (Ramiah and Parthasarathy, 1938). Similarly, an awned wheat variety 'NP 836' was developed by X-ray mutagenesis at IARI, New Delhi (Pal and Swaminathan, 1960). Induced mutations have been used worldwide to improve major crops including rice, wheat, barley, cotton, groundnut and beans. The International Atomic Energy Agency (IAEA) under FAO has catalogued more than **3200 varieties** of plants developed by using mutation breeding.

Most of the crop varieties have a problem of **narrow genetic base** due to domestication and breeding bottlenecks during their development. To solve this problem the genetic base of varieties were broadened using **distant hybridization** or **wide crosses**. India took a global lead in utilizing wild relatives species of crop plants for introgression of novel genes for disease resistance, productivity and quality in sugarcane, wheat, rice and cotton. Sugarcane Breeding Institute at Coimbatore started **inter-species hybridisation** between noble sugarcane (*Saccharum officinarum*) and its wild relative *Saccharum spontaneum* to develop new sugarcane varieties which replaced the 'deshi' *Saccharum barberi* varieties having poor yield and low sugar recovery. Commercial sugarcane variety 'Co 205' developed in this way showed 50% yield advantage and completely replaced the traditional sugarcane varieties in less than 10 years. It was a landmark in the history of plant breeding and the earliest example of using wild species for crop improvement. The process known as **nobilisation of cane**, is a great gift by the Indian scientists to the world sugar economy (Venkataraman, 1938). With sustained breeding efforts the sugarcane acreage in India has increased four-fold from 1.17 to 4.73 Mha, productivity two and a half-fold from 30.9 to 79.9 t/ha and production ten-fold from 36.35 to 376.9 Mt between 1931 to 2018.

Soon after the discovery of *Ph1* locus regulating **chromosome pairing in wheat** (Riley and Chapman, 1958; Sears and Okomoto, 1958), Indian scientists used inter-specific crosses involving *Triticum zhukovskyi* to transfer a large number of genes from wild relatives of wheat (Upadhya and Swaminathan, 1963). The *Ph1* gene was widely exploited globally for transferring genes from wild relatives of wheat and has contributed several billion dollars to the US economy over the last six decades. Indian scientists were among the first to report **cytoplasmic male sterility** in rice and its use in breeding **hybrid rice** using *Oryza sativa f. spontanea*/*Oryza sativa* crosses (Sampath and Mohanty, 1954), and also proposed **clonal propagation** as a means to exploiting hybrid vigour in rice (Richharia, 1962). Both these technologies were widely adopted by China to become a world leader in hybrid rice production. Further, India initiated the **Indica/ Japonica rice hybridization** at CRRI Cuttack to combine high nitrogen response and yield potential with tolerance to insect pests and diseases in Indica rice background (Parthasarathy, 1954). Several varieties were released in different countries based on these crosses, of which Mahsuri released in Malaysia became popular as "Ponni" in Tamil Nadu, and has been used extensively in breeding mega varieties of rice, e.g. Swarna, Samba Mahsuri, Sona Mahsuri, Tapaswani etc. This pioneering technological intervention laid the foundation of breeding strong culm tall, dwarf and semi-dwarf miracle varieties of rice worldwide.

Significant productivity gains were achieved globally by harnessing the hybrid vigour or **heterosis** through use of **hybrid varieties** in maize, pearl millet, sorghum, cotton, castor and many vegetables. The enhanced productivity of these predominantly rainfed crops added significantly to the food, nutrition and livelihood security of the farmers worldwide. The advent of hybrid varieties also revolutionized seed industry in India by triggering private sector investment (Prasanna *et al*., 2001; Hegde, 2010). India is perhaps the only country in the world that has commercially exploited hybrid technology in cotton with the largest acreage and production of cotton in the world. Hybrids in vegetables such as okra, brinjal, chillies, tomato, cauliflower and cabbage have revolutionized the vegetable production in India, making it the second largest producer of vegetables in the world, and enhancing the national nutrition security. Highly productive hybrids have also been developed in coconut, mango, ber and aonla among other fruit trees. Beginning with 'Mallika' and 'Amrapali' (Singh *et al*., 1972), a series of mango hybrids with high productivity, attractive fruit quality, regular bearing and canopy suitable for high-density orchards are a testimony to India's global leadership in mango hybrid breeding. **Tropicalization** and **sub-tropicalization** of **potato** and **grapes** by Indian scientists has a great bearing on the country's horticulture industry. India's **average yields** of potato and grapes are the **highest in the world**. Salinity tolerant **root stocks** for grafting of fruit trees and **mass-multiplication** of banana and sugarcane through **plant tissue culture** have boosted the availability of quality planting materials of elite cultivars.

Scientific breakthroughs in Molecular Genetics and Cell Biology during the second half of the last century have opened up new vistas of precision breeding i.e. **molecular breeding**. These include: discovery of **DNA** as the **genetic material** (Hershey and Chase, 1952); **double helix structure** of DNA (Watson and Crick, 1953), **cracking** the **genetic code** (Nirenberg, 1968), recombinant DNA technique for **cloning of genes** (DNA) (Cohen *et al.*, 1973), reading the code by **DNA sequencing** (Sanger *et al*., 1977), and **creation of transgenic (GM) plants** based on the natural genetic engineering system of *Agrobacterium tumefaciens* (Barton *et al*., 1983; Frawley *et al*., 1983; Herrera-Estrella *et al*., 1983). The ability to regenerate whole plants from a single transformed cell was crucial for the development of GM plants, and Indian scientists were among the first to regenerate whole Datura plants through

anther/microspore culture (Guha and Maheshwari, 1964). Molecular breeding has two major streams: (i) **Marker-assisted selection** (**MAS**) of superior genotypes from the variation existing within the species and (ii) **Genetically modified (GM)** plants for creation of new variation by genetic transformation using purified genes (DNA) from across the species barrier.

Conventional breeding involves phenotypic selection of desired traits such as plant height and seed size in rice and wheat, bigger potato tubers, sweeter strawberries, size and colour of fruits and the overall yield. While these traits are easily selected, difficult to phenotype traits such as disease resistance, drought tolerance, root traits, nutritional and processing quality are very difficult to select. Effect of environment and growth stage of the plant on trait expression makes the selection even more difficult. In contrast, **MAS technology** allows selection of plants with desired traits using DNA from any **tissue** or **growth stage** of the plant grown in **any environment** because DNA is not affected by these conditions. It allows high throughput selection early at the seedling stage and breeders need not to wait till maturity for the phenotypic data. It almost halves the time taken to develop new varieties in comparison to conventional breeding. The first MAS-derived plant varieties were released in 2000, namely disease resistant barley **'Tango'** and low amylose rice varieties **'Cadet'** and '**Jacinto'** developed at Oregon State University and Texas Agricultural Experiment Station, respectively. MAS was soon adopted in India and the first MAS-derived product, a disease resistance pearl millet hybrid **'HHB-67-2'** was released by ICRISAT in collaboration with HAU in the year 2005. First indigenous MAS product, rice variety **'Pusa 1460'** was developed jointly by ICAR-IARI and ICAR-NIPB using marker-assisted introgression of *Xa21* and *xa13* genes for bacterial leaf blight resistance into 'Pusa Basmati 1' (Joseph *et al*., 2004). Beginning with the **decoding of rice genome** (IRGSP, 2005), genomes of more than 50 agricultural plants are now publicly available, which are helping fast track the process of **gene discovery** and **molecular breeding** in these crops. More than 25 MAS-derived varieties have already been released for cultivation in India, e.g. BLB resistant rice 'RP Bio 226', blast resistant rice Pusa 1637, flood tolerant rice 'Swarna-Sub1', flood-drought tolerant rice 'DRR Dhan 50' and quality protein maize 'Vivek QPM 9'. MAS technology is now well adopted by the Indian plant breeders and is limited only by the paucity of validated markers in many important crops due to lack of genetic and genomic information (Singh *et al*., 2016).

GM technology has emerged as a **powerful breeding tool** which will be an essential element of the future plant breeding programme. Nearly a decade after the production of first experimental transgenic tobacco plants in 1983, **'Flavr Savr Tomato'** became the **first GM crop** approved for commercial cultivation in 1994. Today more than **525 transgenic events** in **32 crops** have been released for commercial cultivation on **six continents** of the world, and the GM crop acreage has increased to a record **191.7 Mha** by 2018. Herbicide tolerant cotton, maize, soybean and canola, insect resistant Bt-cotton and Bt-corn, and virus resistant papaya are the predominant GM crops under cultivation today. **Bt-cotton** was the first GM crop approved for cultivation in India in 2002-03. Within ten years of the introduction of Bt-cotton India became the largest producer and one of the largest exporters of cotton in the world. More than 95 percent of India's 11 Mha cotton acreage is now Bt-cotton. It nearly doubled the cotton yield and almost halved the use of chemical pesticides (Shah, 2012). However, GM technology has faced serious opposition from various conservative and environmental groups worldwide expressing biosafety concerns, though no clear evidence of harm has been presented. The mainstream scientific establishments around the world have advocated the judicious use of GM technology, and

more than **107 Noble Laureates** have joined a campaign **supporting the use** of GM crops (Roberts, 2018). **Bt-Brinjal** became the first GM food crop released for cultivation in a developing country after regulatory approval by Bangladesh in 2013. In India however, Bt-brinjal and GM-mustard recommended by the GEAC are awaiting the final government approval. Meanwhile, scientists at MSSRF in Chennai, ICAR-NIPB, ICGEB, DU and JNU in New Delhi, University of Calcutta and Bose Institute in Kolkata have made significant progress in developing GM crop events. MSSRF has identified genes for tolerance to salt and drought stress from mangroves and *Prosopis juliflora*, respectively and transferred these into crop plants. Staple food crop rice has been engineered for high iron/zinc/provitamin A, abiotic stress tolerance, as well as disease and pest resistance (Datta *et al.*, 2012). Elite GM events of pod borer resistant pigeonpea and herbicide tolerant cotton have been developed by ICAR-NIPB using own Bt and CP4 genes, respectively (Ramkumar *et al.*, 2020; Karthik *et al.*, 2020).

Global research on C4 photosynthesis in staple crops like rice and wheat, biological nitrogen fixation to reduce the use of chemical fertilizer, efficient solubilization of phosphorus and potassium by the plants or with the help of bio-fertilizer agents, micronutrient efficient crops, and crops with in-built resistance to diseases and pests for reducing the use of externally applied chemical fertilizers and pesticides are becoming a reality with the help of genetic engineering breeding tools. Efficient bio-fertilizer and bio-pesticide microbial inoculants are also being developed using the power of genetic engineering. Bio-diesel produced from genetically engineered bacteria or algae has great future potential. IPR challenge in the WTO regime makes it imperative that India invests heavily in basic sciences research and develops self-owned crop biotechnologies for its large agrarian population. The research programs need to involve multi-disciplinary networks involving basic, translational and social science disciplines for synergy of actions and scientific prioritization of objectives with enabling policy environment. With this background the NAAS Round Table discussion on 'Regulatory pathways for genome-edited plants' was held in the NAAS committee room at NASC, New Delhi.

2. Genome Editing: A New Powerful Precision Breeding Tool

During the last six years, **genome editing** or **gene editing** has emerged as a **potent plant breeding tool** for creating useful genetic variation by precise DNA sequence changes in the targeted region of the genome using site-directed nucleases (**SDN**) or sequence specific nucleases (**SSN**) (Langner *et al.*, 2018). Developments in targeted mutagenesis started with the use of meganucleases **(MN)**, zinc-finger nucleases **(ZFN)** and transcription-activator-like effector nucleases (**TALEN)**. However, recent developments have provided highly versatile clustered regularly interspaced palindromic repeat (**CRISPR)**-CRISPR-associated proteins (**Cas**) based genome editing systems. Unlike the ZFN and TALEN which are dependent on protein-DNA binding specificity, the CRISPR-Cas system uses RNA-DNA binding for high specificity. A variety of endonucleases are now available under the broad umbrella of CRISPR-Cas systems. Development of CRISPR-Cas9 based genome editing tools has opened up a vast array of possible applications in plant breeding. The basic principle of genome editing is creation of targeted double-strand beaks (**DSBs**) by the SDNs/SSNs like TALEN and Cas9, which then gets repaired by endogenous DNA repair mechanisms. The repair could be either by non-homologous end joining (**NHEJ**) or homology-directed repair (**HDR**). NHEJ frequently leads to small insertion deletion (Indel) in the target DNA thus creating point mutations. In contrast, HDR can be used for precise sequence insertion, deletion or substitution in the target genomic

region by designing a synthetic guide RNA (**sgRNA**) specific to the target gene for editing. Similar to the development of conventional breeding tools summarised above, the CRISPR-Cas genome editing has also emerged from the basic science research. Clustered regulatory repeat sequences were identified first in *E. coli* by Ishino *et al.* (1987) and several other prokaryotes then after, but the fundamental role of CRISPR-Cas system in bacterial adaptive (acquired) immune response against viral attacks was described twenty years later in *Streptococcus thermophilus* (Barrangou *et al.*, 2007). Due to presence of several *Cas* genes it took another five years to work out the exact mechanism how the CRISPR-Cas system creates DSBs in the target DNA. It was shown that CRISPR-Cas system naturally acquires spacer sequences from an invading plasmid or bacteriophage DNA, which ultimately leads to site-specific cleavage and elimination of the plasmid or phage DNA. Independent groups elucidated the role of CRISPR RNA (**crRNA**) in forming a two-RNA structure by base-pairing with transactivating crRNA (**tracrRNA**) and direct Cas9 of *Streptococcus pyogenes* to make the DSB in target DNA (Gasiunas *et al.*, 2012; Jinek *et al.*, 2012). Further, it was found that a proto-spacer adjacent motif (**PAM**) was essential for the recognition of target DNA sequence by Cas9 (Cong *et al.*, 2013). This basic knowledge was crucial for designing a simple two component **CRISPR-Cas9 genome editing** tool with (i) a monomeric DNA endonuclease Cas9, and (ii) a customizable sgRNA. The Cas9 is a bi-lobed protein with a large recognition lobe connected to a small nuclease lobe consisting of two nuclease domains and a PAM-binding domain. The sgRNA is a customizable small non-coding RNA consisting of a fusion of two RNA moieties, namely protospacer-containing crRNA and tracrRNA. The Cas9-sgRNA complex probes the genome for presence of canonical PAM sequence (5'NGG3') and sgRNA complementarity in the PAM flanking sequence. Complementary base pairing allows formation of guide RNA/target DNA heteroduplex followed by double strand cleavage by the Cas9 nuclease (Sternberg *et al.*, 2014).

Although the canonical PAM sequence motif is present widely in all genomes, its essentiality for Cas9 activity limited the spectrum of target sequences to the GC-rich regions. Fortunately, Cas9 from different bacteria have varying PAM sequence requirement thus enlarging the target range. For example, Cas9 from *Staphylococcus aureus* (SpCas9), *Staphylococcus thermophilus* (StCas9) and *Neisseria meningitidis* (NmCas9) require different PAM sequences. In addition, NmCas9 binds to a 24 bp PAM motif conferring greater specificity than the 20 bp PAM for SpCas9. Due to different specificities, *Sa*Cas9 and *St*Cas9 can be used together in one plant cell to target different genomic sites without any interference (Puchta, 2017). New CRISPR nucleases **Cas12a** (**Cpf1**) from *Prevoltella* and *Francisella1*) and **Cas13a** (**C2c2**) from *Leptotrichia wadei* with different PAM specificity have been isolated. Cas12a requires an AT-rich (5'TTTN3') PAM motif instead of the 5'NGG3' for Cas9. Also, a Cas12a modified for genome editing requires only a 42 nucleotide crRNA guide region, as compared to 100 bp for Cas9 and creates DSBs with 4-5 base overhangs as compared to the blunt ends generated by Cas9 (Zetsche *et al.*, 2015). Cas13a can catalyse RNA-guided single-stranded RNA (ssRNA) degradation using two separate catalytic sites in eukaryotic and prokaryotic binding domains. Further, Cas13a requires a protospacer flanking site (**PFS**) instead of PAM to induce a single-strand breaks (**SSBs**), preferentially at the uracil residues. Highly efficient RNA cleavage has been achieved using 22 base crRNA with much reduced off targets as compared to RNAi, making Cas13a-based gene silencing a promising alternative to RNAi. An exciting opportunity is the use of Cas13a for *in vivo* RNA editing (Gootenberg *et al.*, 2017).

CRISPR-Cas based genome editing offers many advantages over other genome modification techniques. It is a simple, fast and the most precise plant breeding tool available to date. All other

plant breeding methods including mutation (natural or induced), recombination (by natural cross-pollination or artificial hybridization), transposon-mediated changes (natural, tissue culture induced or by transformation) and genetic modification through transgenic cerate random changes in the genome, from which the breeder has to select the desired genotype using either phenotypic or marker-assisted selection. In contrast, the guide RNA in genome editing confers target specificity that can be designed based on the target gene(s) and genome of choice. Genome editing does not have the problem of linkage drag which is common in conventional breeding with closely linked useful and deleterious genes, and expression problem due to random integration in the genome and presence of antibiotic or herbicide tolerance genes as selectable markers in the GM crops. Genome editing creates precise and stable mutations which can be easily segregated from the integrated transgenes to develop transgene-free plants. It is highly versatile with diverse plant breeding application: (i) creation of Indels in the protein coding sequence, (ii) creation of indels in the promoter region of the gene (iii) deletion of entire gene or chromosome fragment (iv) gene replacement or insertion of a new gene or allele from the same or related species of the primary gene pool (v) insertion of a new gene from across the species barrier similar to GMO, but at precise location in the genome and free from any marker gene (vi) single base editing (vii) gene silencing by RNA processing, and (viii) epigenetic editing.

Genome editing using CRISPR-Cas 9 in plants was first demonstrated in 2013 using reporter genes in model plant Arabidopsis (Li *et al.*, 2013), rice (Feng *et al.*, 2013) and tobacco (Nekrasov *et al.*, 2013), closely followed by important food crops, namely wheat (Wang *et al.*, 2014), maize (Liang *et al.*, 2014), rice (Miao *et al.*, 2013), tomato (Brooks *et al.*, 2014) and sweet orange (Jia and Wang, 2014). The technology has caught up like a wild fire during the last six years and several useful targeted mutations and gene insertion/deletion have been created in the crop plants (reviews by Langer *et al.*, 2018; Es *et al.*, 2019; Xu *et al.*, 2019). Specific examples of transgene-free genome edited crops include: (**1**) Chlorsulfuron herbicide tolerant maize by targeting acetolactate synthase genes *ALS1* and *ALS2* (Svitashev *et al.*, 2015); (**2**) Broad spectrum (*Xanthomonas* and *Pseudomonas*) bacteria resistant tomato by targeting downy mildew resistance gene *SlDMR6-1* (deToledo *et al.*, 2016); (**3**) Non-browning white button mushrooms by targeting specific polyphenol oxidase (*PPO*) gene (Waltz, 2016); (**4**) Broad spectrum virus resistant cucumber by targeting eukaryotic translation initiation factor gene *Cs-eIF4E* (Chandrasekaran *et al.*, 2016); (**5**) Powdery mildew resistant tomato by targeting mildew susceptibility gene *SlMLO1* (Nekrasov *et al.*, 2017); (**6**) Drought tolerant maize by targeting *ARGOS8* gene, a negative regulator of ethylene response (Shi *et al.*, 2017); (**7**) Bacterial leaf blight resistant rice by targeting sugar transporter genes *OsSWEET11-13* (Oliva *et al.*, 2019).

Genome-editing technology has evolved rapidly over the last seven years as a powerful tool for basic science research as well as molecular breeding applications in agricultural plants. CRISPR-Cas9 system is the most precise plant breeding tool available to date and has become a gold standard for creating desired changes in the targeted regions of genome due of its simplicity, specificity, efficiency and versatility. A wide range of natural and engineered sequence-specific nucleases have enhanced the versatility of the system for plant genome editing and novel applications. Genome editing has been used to enhance plant disease resistance against many pathogens. Disease susceptibility genes and similar negative regulators of useful agronomic traits are prime targets for genome editing. Knocking down of the susceptibility (*S*) genes offers exciting prospects to complement the resistance (*R*) genes

based approach for developing durable disease resistance. Therefore, it is imperative to put in place at the earliest an efficient and effective regulatory system for commercial release of genome-edited plant varieties for the benefit of Indian farmers and consumers.

3. Regulatory Framework for Genome Edited Plants

a. Global regulatory scenario on genome edited plants

Genome editing by site-directed nucleases is a revolutionary new precision breeding tool which has become even more precise and efficient over the last five years so much so that allowing a single base change in the target gene. Many of the products developed through genome editing are indistinguishable from the natural spontaneous or induced mutations and therefore are difficult to monitor and regulate. Therefore, countries around the world are revisiting and modifying their legislation and biosafety regulations for rapid adoption of this powerful technology. For regulatory purpose the genome-edited products have been grouped into the following three categories depending on the nature of joining of the targeted double-strand break (DSB) created by side-directed nuclease (SDN), with or without homology-directed repair (Friedrichs *et al.*, 2019; Ricroch, 2019). (**a**) **SDN1** involves repair of DSB in the target DNA by non-homologous end joining (**NHEJ**) through endogenous repair mechanism without homology-directed repair (**HDR**). Such repairs create small indels at the joining point resulting in gene silencing, knock-out or a change in gene activity. (**b**) **SDN2** involves template-guided or HDR repair of the targeted DSB using a short single-stranded donor DNA. The donor DNA carries one or more small mutations flanked by sequences matching one or both ends of the targeted DSB (known as repair template), allowing desired changes in the target gene. **SDN3** involves template-guided HDR of the targeted DSB using a double-stranded donor DNA, containing a complete gene or even longer genetic element. The ends of the donor are homologous to the two DSB ends (typically over 800 bp each) This allows gene replacement or insertion of a new gene from the same, related or a distant species at the target site similar to the genetically modified organism (**GMO**).

The biosafety regulations for genome edited plants in different countries can be grouped into four categories: (i) same as GM plants, (ii) less stringent than GM plants, (iii) still under discussion, and (iv) no public discussion as yet. In 2018, the European Union Court of Justice (**ECJ**) ruled that genome-edited organisms must be regulated in the same manner as GMO, even though the products of chemical and radiation mutagenesis are exempt from biosafety regulation. Apart from the EU, New Zealand is the only other country which regulates genome-edited organism (**GEO**) in the same manner as GMO. Both EU and New Zealand regulations are based on court rulings and not by scientific establishments and have negative impact on innovations in this field as major agricultural companies have moved out of Europe. Following the ECJ decision a study has been commissioned by the European Commission in 2019 to examine the question of enforcement of the EU regulation, risk assessment framework developed by European Food Safety Authority (EFSA), ethical and social aspects and applications of genome editing technology through consultations with all stakeholder, including scientific organisations, industry, farmers, NGOs. The study is likely to be completed in April 2021. Governments of Norway, Switzerland and UK are considering separate laws for the regulation of GEO, particularly the Norwegian Biotechnology Board has proposed exemption of SDN1 products from biosafety regulations. Several major agricultural

countries, namely Argentina, Australia, Brazil, Canada, Chile, Israel, Japan, Peru and USA have exempted from regulation certain categories of GEO which do not possess any foreign DNA. Countries like India, Bangladesh, Philippines, Indonesia, Kenya, Nigeria, Paraguay and Uruguay are actively discussing separate bio-safety regulations/exemptions for GEO and GMO. At the same time some big countries like China, Russia, Mexico and South Africa have no public discussion on this issue as yet (Schmidt *et al.*, 2020).

b. State of regulating genome-edited plants in India

A roundtable discussion involving wide range of stakeholders including Fellows of the Academy, representatives from seed industry, progressive farmers and other relevant experts was held under the Chairmanship of President NAAS, Dr T. Mohapatra, and past Presidents of NAAS, Dr R.S. Paroda and Dr R.B. Singh, on the potential of emerging powerful genome editing tools in solving the problems of entrenched low farm productivity, malnutrition and hidden hunger in a large section of Indian population. This was followed by a focussed discussion on the *'Draft Document on Genome Edited Organisms: Regulatory Framework and Guidelines for Risk Assessment'* circulated by Department of Biotechnology (DBT), Government of India, inviting comments from the researchers, institutions and other stakeholders.

In the past six years, genome editing in crop plants has become one of the most intensively researched areas in countries that are keen on developing and maintaining their strength in Science and Technology. India however, is much behind countries like China and USA, both in terms of new developments and in use of this technology because of lack of policy directions or decisions enabling its usage despite availability of trained manpower. No significant research has been carried out in our country on describing any new CRISPR-based system or on developing and testing any newly engineered SDNs for more efficient mutagenesis. Sooner the policy environment enables use of the technology, better it would be in dealing with the host of biotic and abiotic stresses faced by Indian agriculture while facing the ever increasing challenges to sustainable food and nutritional security of the country. This will be in the long-term interest of India, much like the technological interventions in the last century that enabled India tide over the 'ship to mouth' existence to become a food secure nation. ***Any biosafety regulations on genome editing must not be overbearing and restrictive to research and development, rather it should facilitate applications of genome editing for product development and reaching the unreached for the socio-economic and ecological benefits.***

4. Recommendations for the Regulation of Genome Edited Plants in India

1. Having signed the Cartagena Protocol within the realms of the Environmental Protection Act (1986), India's regulatory systems are already in place to deal with any use and environmental release of agricultural crop plants possessing genetically engineered events in their genome. In the case of GEO too, in line with the EPA (1986) along with the existing Seed Act (1966), the new plant varieties developed through genome editing need to go through the regulatory processes where required, for risk analysis of biosafety and environmental safety, so that the technology applications are in compliance with the Protocol. Thereby, facilitating achieving the SDGs with all the socio-economic and ecological benefits, while the country takes strides towards the cherished goal of becoming a 5-Trillion dollar economy.

2. Genome editing is a powerful new breeding tool with unprecedented precision and pace for developing new plant varieties, animal breeds and microbial strains with useful traits for a sustainable low-input high-output agriculture. **However, being different in nature and use in the country in relation to any consequential risks to environment or biosafety of the resultant plants, animals, human somatic cells and microbes, any policy decision on use of the technology has to be specific and unambiguous to each of these products. Accordingly, the NAAS representing a national think tank for the diverse agricultural sectors felt that the Guidelines need to be prioritized and developed separately for each sector while it took up the discussions on the draft document on GEO. It was unanimously decided to focus on regulatory requirements and guidelines on genome editing in plants only in the first phase of regulation.**
3. Since the current draft DBT guideline has clubbed together plants, animals, and human somatic cells, it is likely to lead to confusion and delays in approval of agricultural products due to the nature and complexity of different organisms. **A science-based differentiated and disaggregated approach must be followed and hence, there is a need to have separate guidelines for genome edited plants**.
4. The proposed categorisation of genome edited organisms in the DBT draft as GEd1, GEd2 and GEd3 should be replaced by internationally accepted and clearly defined SDN1, SDN2 and SDN3 for plants.
5. Although the genome edited product development usually starts with genetic transformation of the cell, certain categories of the final products (SDN1 and SDN2) developed through genome editing are free from foreign DNA and indistinguishable from the products developed through conventional breeding utilising natural genetic variation or induced mutations. Hence, **it is not necessary and also not scientifically possible to regulate these products under Rules 1989 of the Environment (Protection) Act 1986.**
6. Since, the genome editing is a precise and targeted mutagenesis tool and the final products of genome editing using SDN-1 and SDN-2 approaches do not carry any vector DNA and are similar to the products of spontaneous or induced mutations, these should be exempted from regulation and risk assessment. Provision for such exemption exists under rule 20 of the "Rules for the manufacture, use, import, export and storage of hazardous microorganisms/ genetically engineered organisms or cells, Rules 1989". Also, there is a precedence wherein exemptions have been granted to rDNA pharma products under the said rule 20, as per the Gazette notification G.S.R. 616 (E), dated September 20, 2006 based on the report of the Task Force on Recombinant Pharma under the chairmanship of Dr R.A. Mashelkar (2005).
7. As the initial generation of genome edited plants involves rDNA techniques, their registration with IBSC with information to RCGM will continue. However, for commercial release of the final product, which is devoid of any foreign DNA, the guidelines for evaluation of **trait efficacy and field performance shall be carried out as per the ICAR-AICRP guidelines or any other extant procedure for evaluation, release and notification as per the legislations for seed quality regulations (Seeds Act 1966, Seeds (control) order 1983, Seeds Rules, PPVFRA Act 1986, National Seed Policy and the proposed New Seeds Bill).** Hence, the data requirement for molecular and phenotypic equivalence as given in the draft DBT guidelines for all GEOs is unnecessary and restrictive. A proposed flow chart for development and release of such genome-edited plants is given here **(Figure 1).**

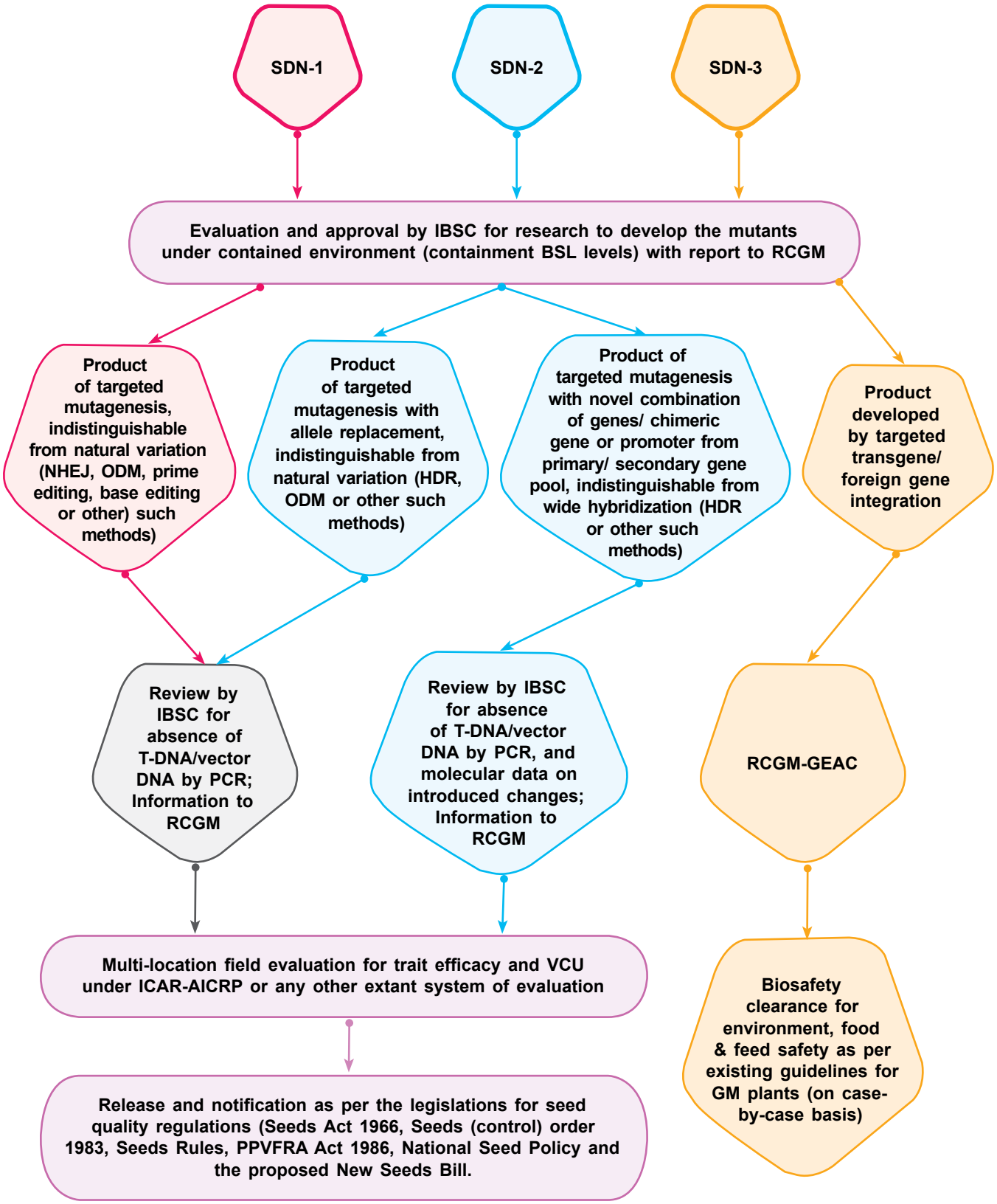

Fig. 1. Regulatory pathway for the release of genome-edited plants

8. The SDN 3 type of genome editing can be used to insert transgenes in a specific region of the genome and therefore, it avoids the shortcoming of random insertion of transgenes like transgene silencing, disruption of endogenous genes and position effects. Therefore, the rules governing the biosafety and release of the SDN 3 products should be less stringent as compared to the conventional GMO in terms of data requirement for substantial equivalence.
9. Genome edited plants involving replacement of an existing gene with superior alleles from the same species or primary/secondary gene pool with known protein function should be categorised as SDN 2 type product.
10. The foods derived from the SDN1 and SDN 2 categories should not be treated as "genetically engineered or genetically modified food" under the Food Safety and Standards Act, 2006.
11. The DBT draft document on GEOs overemphasises the off-target effects for plants. In case of plants, off-target mutation(s), if any can be easily bred out by crossing and selection.
12. The national regulatory system on genome edited plants should be in harmony with countries such as Australia, Brazil, Canada, China, Japan and USA to facilitate smooth international trade of genome edited products, and for effective exchange and sharing of genetic material for research and development.
13. The group felt that tremendous benefits and safety of the new genome editing tools should be communicated to general public, policy makers and farmers in a simplified manner. Particularly the message should be conveyed that these products are as safe as those developed by conventional breeding. Most importantly, these tools allow precision and rapid delivery of the desired products.
14. Return from investments in biotechnology research is very high. Therefore, public and private sectors in India should develop a joint strategy for product development through enhanced investment with a view to ensure affordable access to improved technologies for all farmers, small or large. This is also essential for self-reliance (*Atmanirbharta*) in harnessing the upcoming billion dollar genome editing industry.
15. To achieve the zero hunger challenge of the United Nations, and to meet the Sustainable Development Goals 2030, especially SDG 1 and SDG 2 (eliminating poverty and hunger, respectively), we must significantly enhance the small farm productivity and double farmers' income, "**leaving no one behind".** Such a transformation will be possible only through the intelligent and intensive applications of new technologies and innovations such as genome editing.

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