PLANT SCIENCE TODAY
Vol 12(4): 1-19
https://doi.org/10.14719/pst.7530

eISSN 2348-1900

REVIEW ARTICLE

Plant genetic engineering, molecular farming and biosafety regulations in India

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Received: 30 January 2025; Accepted: 18 July 2025; Available online: Version 1.0: 27 October 2025; Version 2.0: 05 November 2025

Cite this article: Maurya AK, Saksena YP, Sheetal, Kaushik B, Gaur K, Banerjee S, Bachwat T, Kulkarni G, Sinha D. Plant genetic engineering, molecular farming and biosafety regulations in India. Plant Science Today. 2025; 12(4): 1-19. https://doi.org/10.14719/pst.7530

Abstract

Molecular farming is a biotechnological approach that modifies plants or other organisms to produce desired and valuable products such as proteins, chemicals, or pharmaceuticals. These products are challenging to get or expensive to manufacture using classical biotechnological methods. The molecular production system primarily involves plants, animals and microorganisms such as algae, yeast and bacteria. The plants are often referred to as "bioreactors". Several biotechnological methods are applied, such as modifying plant-based expression systems involving nuclear or chloroplast genomes or enhancing plants' Heterologous Protein (HP) accumulation. The advantages of molecular farming are seen in their cost-effectiveness, scalability, faster production and safety. These bioreactors are known to produce antigen proteins used in vaccine development and nutraceuticals like omega-3 fatty acids, vitamins and nutritional benefits. The generated vaccines have become popular as edible vaccines. However, challenges and concerns associated with such products and methods as Genetically Modified Organisms (GMOs) having such potential are controversial, with concerns about environmental impact, safety and ethical issues. Also, molecular farming products are questioned for their yield, efficiency, purity and stability. This has led to the approval of only one non-food GM crop for cultivation in India despite several crops being ready to be launched. On the other hand, in terms of regulatory setup, India has a robust system and strict regulatory frameworks to ensure the safety of GMOs and the products they produce. India possesses adequate provisions under legal regimes, i.e., the constitution and various statutes that incorporate desired provisions as per national needs or international agreements on biosafety, the security of the environment, or public health. Despite issues, molecular farming holds great promise, especially in providing more affordable and scalable alternatives to traditional pharmaceutical manufacturing methods. As technology improves and regulatory hurdles are addressed, molecular farming could become an even more important tool in medicine, agriculture and environmental sustainability. The intended review aims to compile and analyze the available, relevant, legal-scientific and policy information about genetic engineering and products derived from such technologies, with special reference to products derived from molecular farming. The review includes two parts: the first covers molecular farming and genetic engineering-derived products and the second covers legal aspects related to their ethical consequences.

Keywords: bioreactor; bio-safety; gene; genetically modified organisms; molecular farming

Introduction

Plant Molecular Farming (PMF) or Molecular Farming (MF) produces genetically engineered proteins, ranging from industrial proteins to pharmaceuticals and other critical secondary metabolites, using plants as host organisms. This encompasses the entire process of cultivating, gathering, transporting, storing and further treating the extraction and refinement of the protein (1). The technology relies on the genetic modification of plants, a breakthrough initially proven in the 1980s (2). The PMF technique has been employed and is effective in generating protein products from the initial stages of higher plant development (3). The inception of PMF started in the 1980s when the marker gene for β-glucuronidase (GUS) was effectively introduced into higher plants by transformation (3, 4). Subsequently, β-glucuronidase emerged as a triumphant commodity in PMF, along with two industrially valuable enzymes, avidin and trypsin (5 - 7). The PMF concept was expanded in 1986 to produce human growth hormone (hGH) in genetically modified tobacco and sunflower (8). Subsequently, the manufacture of an IgG1 antibody in genetically modified tobacco was accomplished years later (9). Human antibody production in plants was reported in 1988 (10). The idea of utilizing plants to produce vaccines, which became popular as edible vaccines, was presented by Dr. Charles Arntzen during the 1990s (11). Several efforts on the same line of

investigations were undertaken in the subsequent years to develop more edible vaccines. In 2002, a study indicated the potential of plant virus-based expression systems as adjunctive oral boosters for rabies vaccinations (12). In 2004, a study was conducted on creating a prospective edible vaccination against hepatitis B and HIV using transgenic tomato plants (13). In 2007, edible vaccinations against malaria were suggested using transgenic tomato plants expressing antigenic types (14). In the subsequent year, an edible rabies vaccine using maize was proposed (15). The studies have been found to have potential in combating viral infections (12). In 2006, *Nicotiana benthamiana* cell cultures that had been genetically modified to be stable were utilized to manufacture the first chicken Newcastle disease vaccine that could be injected, which was approved by the USDA (16). An enzyme, human β-glucocerebrosidase, used for Gaucher disease type I, was developed by the stable transformation of carrot cell suspension culture in 2012. Protalix Biotheraputics Inc. developed this product, which was approved as Elelyso® by the FDA and licensed to Pfizer (17).

The successful production of plant-based recombinant antibodies showed that plant systems possess more advantages over other platforms, like mammalian cell cultures (18). First, plants are intrinsically safe as they do not support the replication of human infections, leading to a minimal presence of pathogens and a low likelihood of contamination connected to the production process (19). Furthermore, cultivating plants is uncomplicated as it does not require a sterile setting. Healthy plants can depend on their defense to prevent the intrusion of infections (20). Moreover, cost-effective and well-defined fertilizer solutions are suitable for cultivation (21), in contrast to the costly media needed for mammalian cell cultures (22). A further benefit is that the production of recombinant proteins in plants can be accomplished in approximately 8 weeks (23, 24) after obtaining the relevant DNA sequence, usually by temporary expression facilitated by the infiltration of *Agrobacterium tumefaciens* and/or viral vectors (25-28). In addition, plants can carry out post-translational changes like mammalian cells. This makes them more advantageous than prokaryotic systems when producing complicated proteins like monoclonal antibodies or membrane proteins (17). Considering its immense advantage, this review attempts to highlight PMF from a technological and commercial point of view. Efforts have also been made to highlight the legal aspects with special reference to India, compared with the European Union (EU) and United States of America (USA), associated with PMF techniques.

Methodology used

The proposed review aims to aggregate and evaluate the pertinent legal, scientific and policy knowledge about genetic engineering and its related products, with particular emphasis on those originating from molecular farming. The review is divided into two sections: the first section focuses on molecular farming and products developed from genetic engineering. In contrast, the second section examines the legal considerations related to the ethical implications of these products. The review integrates legal-scientific information by sourcing papers from online resource repositories, including Google, Google Scholar, PubMed, Scopus, Web of Science and specific journal platforms. The information was collected using keywords such as genetic engineering, Molecular farming, biotechnology-based products, recombinant DNA technology, living or genetically modified organisms, international laws related to LMO/GMO, Indian laws about LMO/GMO and Biosafety, among others. The review paper attempts to incorporate recent documents as priorities, except for the pioneering work, which warrants due mention in the manuscript.

Methodologies of plant molecular farming

Presently, there exist four methodologies for synthesizing proteins from plants: (1) the incorporation of genes into the nucleus of a crop species cultivated in either field or greenhouse settings, (2) the introduction of genes into the plastids of a crop species, (3) the transient introduction of genes into a crop species and (4) the insertion of genes into a plant species grown hydroponically, facilitating the secretion of protein into the medium for collection (29). Fig. 1 depicts the different biotechnological approaches used in molecular farming.

Stable nuclear transformation

Transformation introduces a modified DNA or RNA molecule into cells to make the desired protein. The transformation system will profoundly influence the end outcome, equally as the host species. Transformation may demonstrate either stability or impermanence. Stable transformation is a procedure through which DNA is incorporated into nuclear or plastid genomes through biolistic processes by co-cultivating plant cells with genetically modified *Agrobacterium*. The genetic material is subsequently integrated into the target organism's genome, becoming a permanent constituent (30). However, stable transformation has some drawbacks, such as being time-consuming and expensive, requiring several years to restore cell lines and low proteins. The nuclear transformation strategy can be subdivided into two categories.

Non-biological gene transfer

Particle bombardment or biolistics method: Particle bombardment, or biolistics, is a commonly utilized method for genetically modifying plants and other organisms. Numerous metal particles enveloped in DNA are directed toward cells or tissues using a biolistic apparatus or gene gun. The DNA is liberated from the particles that become ensnared within the cells and a portion of it may be irreversibly incorporated into the host chromosomes (31). The particle bombardment or biolistic method was first used to deliver foreign genes into target plant cells using a gene gun machine in 1987, leading to the generation of transgenic plants (32), for example, rice (*Oryza sativa*) (33), maize (*Zea mays*) (34) and wheat (*Triticum aestivum*) (35). One potential drawback of biolistics transformation is that the resultant plants may include several intricate inserts susceptible to recombination and silencing (36). The biolistics method imposes an additional limitation by necessitating the adaptation of particle bombardment procedures for each target tissue, which involves altering critical factors like particle size, distance from the target material and helium pressure (37) (Fig. 2).

Biological gene transfer

Gene transfer across organisms is an inherent mechanism that generates diversity in biological characteristics. This fundamental principle is the basis of all endeavours to enhance agriculturally significant species through conventional agricultural breeding methods or molecular biology techniques (38). The genetic modification of plant

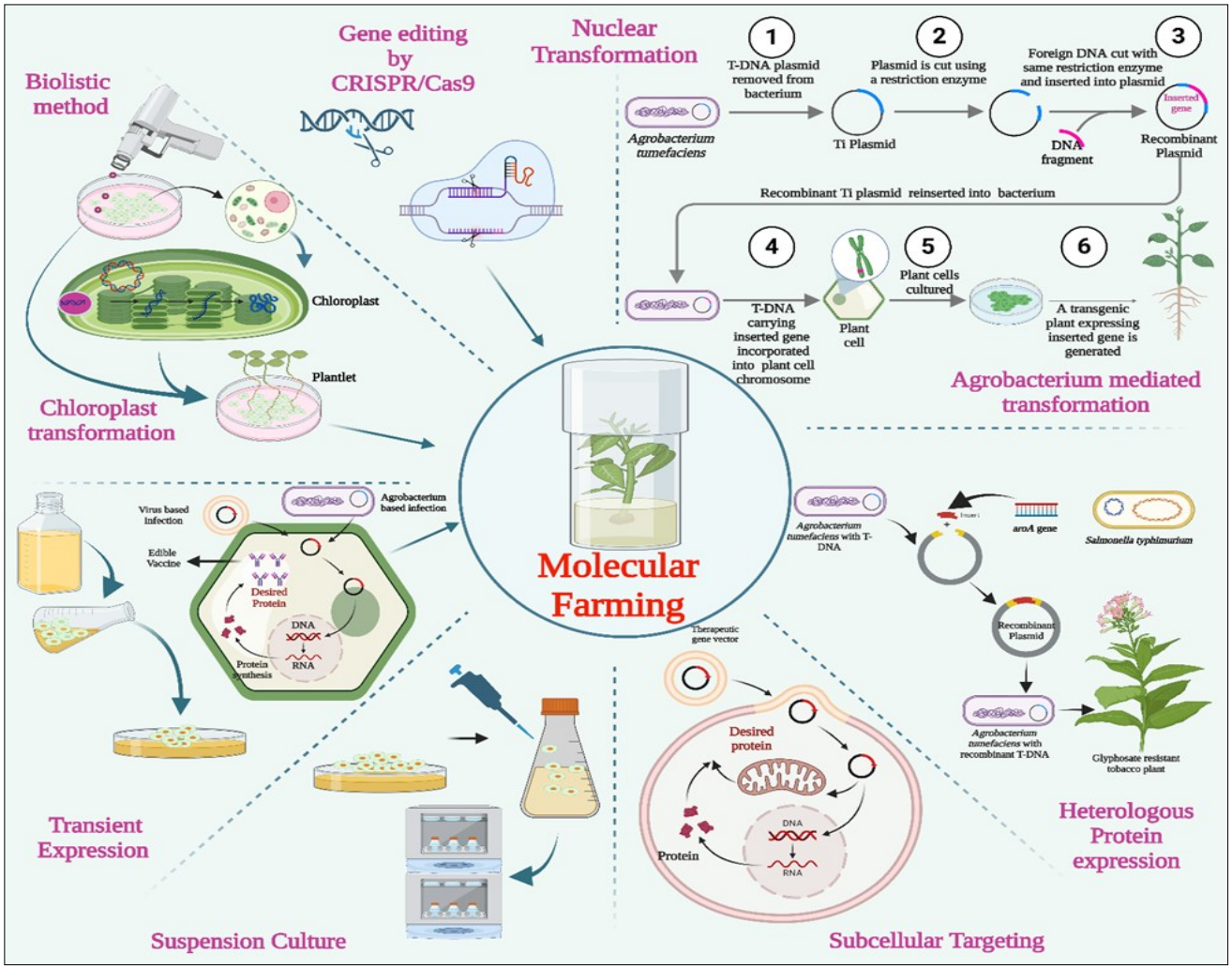

Fig. 1. Diagram depicting the different biotechnological approaches used in molecular farming.

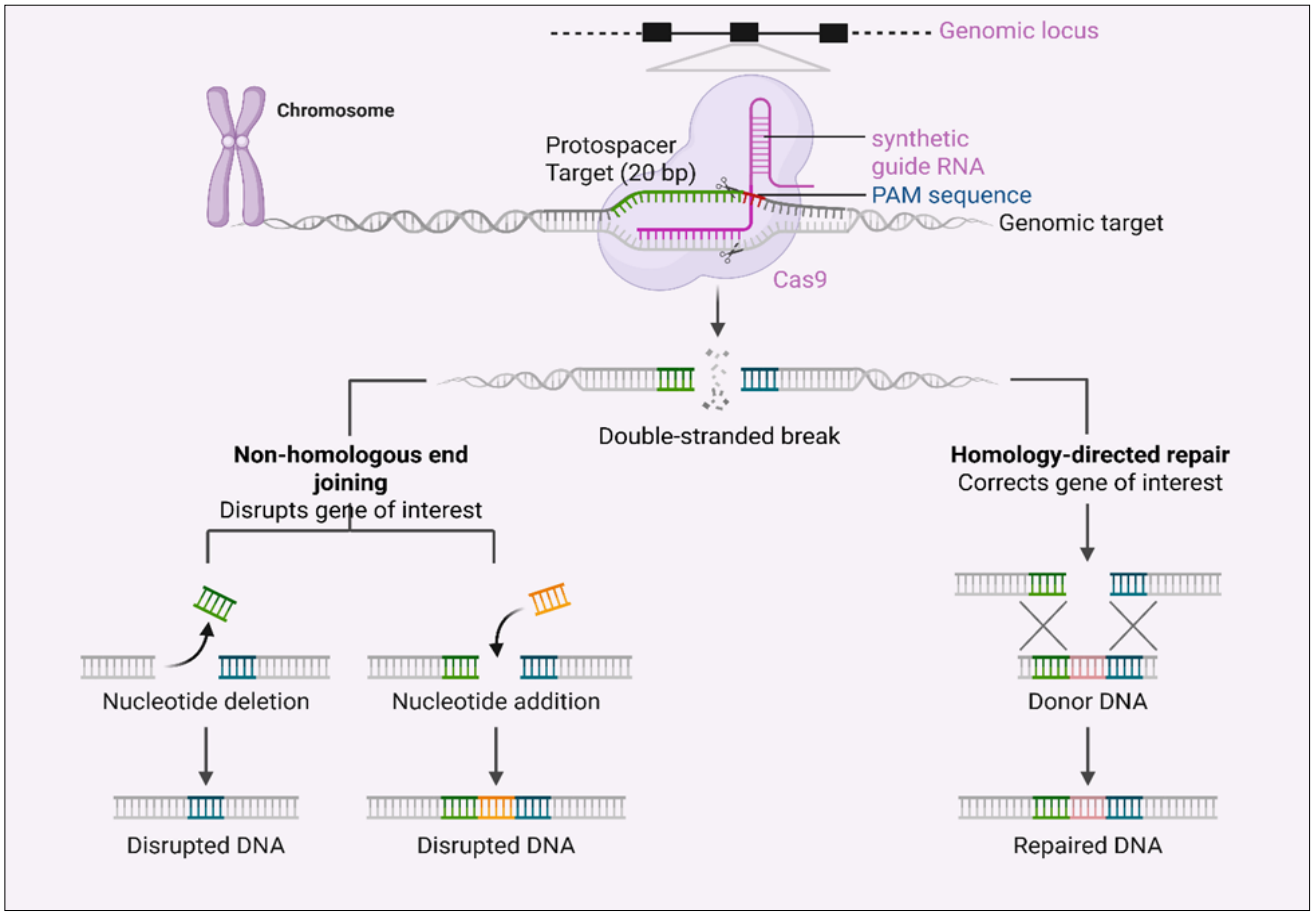

Fig.2. Diagram showing the components and mechanism of CRISPR-Cas9 technology.

cells by *Agrobacterium* entails the transfer of a segment from a substantial tumor-inducing (Ti) or rhizogenic (Ri) plasmid, found in *Agrobacterium*, into the plant nuclear genome (Fig. 2).

Chloroplast transformation: Chloroplast transformation is an ecologically conscious plant genetic engineering method, as it limits the spread of transgenes to other plants and decreases the harm caused by transgenic pollen to unintended insects. The polyploidy of the plastid genome enables chloroplast transformation to include many copies of foreign genes per plant cell, leading to much increased foreign protein synthesis (39). Chloroplast transformation vectors employ two targeting sequences to enclose the foreign genes and introduce them into the organelle genome at a specific and specified site using homologous recombination (40). This leads to consistent transgene expression across different transgenic lines and removes the 'position effect' observed during the nuclear transformation phenomenon in plants. Using the chloroplast transformation technique, agronomic traits like photosynthetic performance, various biotic and abiotic stress resistance and nutritional content have been improved in plant species (41), along with many biopharmaceuticals, industrial enzymes, antibiotics and proteins have been produced (42). The transformed chloroplasts are selected and subsequently regenerated into plants. The chloroplast transformation system offers three key advantages over the nuclear expression system: (i) the higher accumulation of protein products synthesized by the transgene, (ii) prevention of gene pool contamination and (iii) stable integration into the chloroplast genome. Since chloroplast is inherited maternally from a female parent, the risk of transgene transfer by cross-pollination is reduced in the environment (43). Compared to nuclear transformation, where the random integration of transgene occurs, homologous recombination achieves precise transgene integration in chloroplast transformation. Chloroplast transformation has been successfully done in tobacco, cabbage, wheat, eggplant, lettuce, Arabidopsis and carrot (44 - 47). The chloroplast transformation expression system has been utilized to synthesize target proteins and medicines (48, 49). For example, Exendin 4, an analog of Glucagon-peptide hormone used as an insulin substitute (50); Human Interferon gamma (51); E7 antigen as a vaccine against papillomavirus (52); Human Coagulation factors FVIII (53); Envelop protein domain III (EDIII) against dengue (54); CTB-insulin fusion protein in tobacco, (55), the human immunodeficiency virus (HIV-1) Gag (Pr55gag) polyprotein expression, a principle HIV vaccine candidate in tobacco (56), the p24 and Nef antigens expression, an HIV vaccine candidates, as in tobacco and tomato as fusion protein product (57) and, the human papillomavirus type 16 (HPV16) L1 structural protein expression, a candidate for cervical cancer vaccination in tobacco (49) (Fig. 2).

The chloroplast technology is used for the synthesis of enzymes and vitamins; for example, cell wall degrading enzymes of *Thermobifida fusca* produced in tobacco chloroplast help degrade cellulose and hemicelluloses actively during the conversion of plant biomass to sugars (58), Beta-Glucosidase from *Aspergillus niger* and Cellulase from *Thermotoga neapolitana* expressed in tobacco by transforming its chloroplast via bgl1 gene and celA/B genes respectively (59). Two genes, Toc cyclase and Gamma-Toc Methyltransferase, were successfully expressed in lettuce and tobacco chloroplasts to improve their vitamin E content (60). Chloroplast is also involved in other anabolic processes like fat, purine and hormone synthesis (61, 62).

Transient expression

Transient gene expression (TGE) is an excellent and effective tool for investigating the roles of gene products in a short duration. The *Agrobacterium* infiltration method is generally preferred for plant transformation to temporarily introduce foreign genes into numerous target species (63). These are obtained through transitory, high-quality transcription of DNA sequences that may not be integrated into the plant genome. TGE techniques in plants were developed concurrently with stable transformation approaches in the 1980s involving *Agrobacterium tumefaciens*-mediated transformation or direct gene transfer via chemical agents (such as polyethylene glycol (PEG) treatment) or physical techniques such as biolistics (64). The recombinant protein products are transiently expressed and obtained in the ER of *N. benthamiana*, such as Human Growth Factor (HGF), Butyrylcholinesterase, Interferon-gamma, Glycoprotein subunit 1, HIV envelope protein subunit and Dengue virus non-structural protein 1 (65 - 68). It was reported that agroinfiltration was used in stable plant transformation of *N. benthamiana* to produce immunogens for vaccine developments, allowing expression of hemagglutinin-neuraminidase glycoprotein of Newcastle disease virus (69). Pembrolizumab and nivolumab, the first two US Food and Drug Administration (USFDA) approved monoclonal antibodies targeting programmed death-1 (PD-1), used against cancer, have been transiently expressed and produced in *N. benthamiana,* which may contribute to the cost of cancer therapeutics (70, 71).

Plant cell suspension culture

Plant cell suspension culture (PCS culture) is a plant-derived desired product production platform that integrates whole-plant, microbial and animal cell culture systems. It facilitates rapid in vivo production, secretion of expressed protein, or synthesis of desired chemicals in a culture medium. PCS culture simplifies the production process and purification. It contributes to cost reduction by eliminating the need for synthetic media as required in traditional bioreactors, along with enhanced product safety, substantial yield and post-translational changes and the capacity to produce correctly folded and assembled multimeric proteins (72) such as recombinant glycoproteins, which are not possible in chloroplast transformation. There are several examples of CS culture-derived products, such as pharmaceutical proteins, including antibodies in tobacco (73), human interleukin (IL)-2 and IL-4 (74) and human granulocyte colony-stimulating factor (hG-CSF) (75,76). The cell suspension culture based on tobacco led to the development of the Newcastle disease virus (NDV) vaccine, the first vaccine for veterinary application developed by Dow AgroSciences (Indianapolis, IN, USA), which got approval from the US Department of Agriculture (USDA (77). Similarly, Protalix Biotherapeutics (Carmiel, Israel) used cell suspension cultures of carrots to generate recombinant human glucocerebrosidase for treating Gaucher's disease (78). The major limitation of PCS culture is the relatively low protein yields (72) (Fig.2).

Strategies for increasing heterologous protein accumulation in plants

Promising augmentation strategies have been adopted to overcome the problem of low protein yield in PCS culture.

Boosting transcription

The process of transcription is well coordinated and regulated at various levels. Several unique sequences like promoters, enhancers, silencers, polyA tail and introns play key roles in transcriptional regulation (79). These sequences have been utilized to enhance HP production in plants. A robust constitutive promoter can modify a transgene's mRNA abundance at the transcriptional level, influencing the protein accumulation within the cell (80). Promoters of 35S CaMV, actin1 gene from rice and ubiquitin1 gene from maize are widely used to boost transcription owing to their strong constitutive nature (81, 82). Transcription can be improved by adding an extra duplicated promoter element or by creating new synthetic promoters that include the active sequences of many well-characterized natural promoters (83). Among all the known terminators utilized for increasing the transcriptional rate, like ADH, UBQ5 and NOS, GUS expression was increased by the heat shock protein 18.2 (HSP) terminator in *Arabidopsis thaliana* protoplast (84). Experimental evidence suggests that the terminators of several Arabidopsis genes transfected Arabidopsis T87 protoplasts, but the heat shock protein 18.2 (HSP) terminator was found to be the most effective for increasing gene expression levels in *Arabidopsis* plants (84), tomatoes (85) and lettuce (86), *N. benthamiana*, eggplants, hot peppers, melons and orchids (87). Compared to a natural single terminator, such a double terminator system enhanced the expression level. When the HSP terminator was combined with the extensin terminator to create double terminators, it resulted in higher expression of GFP protein in lettuce, tomato, melon and orchid (88). Sometimes, gene expression is enhanced by introns by increasing the steady-state level of mRNA. This was found effective by inserting within the luciferase coding sequence, which led to significantly increased luciferase activity in transgenic barley (89). Eliminating cryptic splicing sites and mRNA destabilizing elements that enhance RNA stability and employing viral suppressors to avert post-transcriptional gene silencing are strategies for augmenting transcription (90). The artificial transcriptional system has also been in tobacco by fusing promoter FM'M-UD and terminator 3PRt and increased expression of reporter genes was observed in this promoter compared to the ubiquitously used CaMV promoter (91). Successful production of anti-HIV-1 monoclonal antibody was obtained in maize when the rice GluB-1 promoter was combined with intron-1 the maize ubiquitin1 promoter (92).

Another organ-specific or inducible-expression strategy is adopted when the transgene does not express the protein or is unsuitable for host cells. Sometimes, the protein expressed by transgenes is unsuitable for the host cells (93). For example, vaccine antigen HBsAg M, murine single-chain variable fragment (scFv) G4 and human interferon-α were obtained by using a regulated promoter system (94). The transcription can be further increased by enhancing promoter activity by inserting a duplicate enhancer region into the CaMV 35S promoter (95). Another way to boost transcription is by combining designed novel synthetic promoters with the most active sequences of natural promoters. Polyadenylation strategy is another way out where a poly(A) tail is added to mRNA that influences the stability of transcripts; for example, the *nopaline synthase* (*nos*) gene present in the Ti-plasmid of *A. tumefaciens* showed efficient mRNA formation in both transiently and stably expressed transgenes (93). Sometimes, introns assist gene expression by increasing the steady-state level of mRNA in plants (96), as seen in the coding sequence of the transgene luciferase, markedly enhancing luciferase activity in transgenic barley plants (89). In addition, removing cryptic splicing sites and mRNA destabilizing elements (97) enhances RNA stability and employing viral suppressors of silence to avert or reverse post-transcriptional gene silencing has also been utilized to elevate transcription levels (90) (Fig.2).

Boosting translation

Enhancement of translation efficiency is achieved by adopting two complementary Strategies, such as increasing the translation rate and reducing the degradation rate of mRNA. This solves the problem of overcoming the low protein yield observed in PMF. The farmer approach involves the use of a 5' untranslated leader sequence of tobacco mosaic virus (TMV) RNA, Potato virus X (PVX) RNA, or rice seed storage-protein (SSP) genes, leading to increased protein expression (98). Gene silencing is reported to occur during HP expression at transcriptional and post-transcriptional levels and "transgenes", when present in multiple copies, are prone to get silenced and leading to a negative effect on boosting translation. Encasing transgenes with matrix attachment region (MAR) sequences has demonstrated a reduction in gene silencing, resulting in either unchanged or enhanced expression (90), which functions by inhibiting the production of antisense transcripts from adjacent endogenous genes into the transgenes. Stacking multiple copies of enhancer from CaMV 35S, using synthetic promoter with natural promoter (CaMV 35S, the *Agrobacterium* Ti plasmid mannopine synthetase promoter) (99, 100) or use of the AUG translation initiation codon and co-expression with protease inhibitors (90) or by altering codons within the gene sequence to improve recombinant protein expression (101) are additional strategies applied for boosting translation (Fig.2).

Subcellular targeting

Targeting HP to the subcellular compartment can be the right Strategies for obtaining high protein accumulation (93). A gene product is an HP isolated from a species different from the receiving organisms expressing it and such proteins don't exist in analogs that are closely similar in donor and receiver species. The production of HPs in plants aims to produce desirable proteins to alter the characteristics of the plant itself (102). It was found that the novel vacuole-targeted determinant could find the recombinant protein (r-proteins) in the vacuole in high concentrations and help purify the sugarcane juice (103). Compared to the vacuole, targeting protein accumulation to the endoplasmic reticulum (ER), chloroplast, or apoplast can be advantageous for less proteolytic degradation and more assisted folding in these compartments (104). Such targeting is achieved by the coordinated action of the ER and Golgi complex, which are involved in the secretory pathway (105), or by signal sequences that can retain the protein in specific compartments (106). Targeting proteins to the apoplast is very popular in hairy root

cultures as it eliminates a few downstream processing stages and the target protein is directly secreted in the media (107). The native extensin secretory signal, when fused with the target gene, resulted in increased secretion of proteins in the rice suspension media (108). Interferon-gamma, specific antibodies, antigens and growth hormones were secreted into the culture media (109, 110). Many recombinant therapeutics have been targeted and accumulated in the ER due to glycosylation patterns and improved folding in plant species like *Arabidopsis*, tobacco, rice and potato. Protein subunits against the Dengue, Ebola, HIV and Yellow fever viruses have been transiently expressed in the ER before their recovery (69, 111 - 113). Chloroplasts have also been utilized to target proteins (114). When targeted to the chloroplast, the recombinant HPV-16 E7 protein fused with an anti-lipopolysaccharide factor exhibited 27-fold higher production (115) (Fig.2).

Fusion protein-based Strategies

Heterologous protein expression in non-native hosts often leads to lower yield due to their aggregation, incorrect folding and proteolysis, affecting solubility and stability (104). To overcome this issue, the fusion protein-based approach boosts transgene protein production in plants (116). The entrapping and downstream processing for recovery are ensured by the peptide or protein tags that enhance the protein accumulation rate in specific organelles, such as vacuoles (117). For example, Zera, ELP, HFBI and AGP are a few of the tags widely utilized to increase the solubility of several recombinant proteins (118 - 121) and facilitate the isolation and purification of target proteins (122). Zera is a proline-rich N-terminal domain originating from the maize storage protein y-zein, possessing self-assembling and protein bodybuilding capabilities (123). A protein body is an ER or vacuole-derived vesicle that can accumulate many proteins (124, 125). The amalgamation of Zera with target proteins has demonstrated the ability to engender protein bodies that promote the steady accumulation of recombinant proteins at elevated levels (107). Zera has been fused with calcitonin, epidermal growth factor (EGF) and vaccine candidates (111, 124, 126). Fusing F1-V hybrid vaccine antigens with the Zera tag in *N. benthamiana*, *Medicago sativa* (alfalfa) and *Nicotiana tabacum* NT1 cells resulted in the expression of at least three times more recombinant proteins in cells expressing the Zera-fused F1-V antigen compared to the control (127). The Elastin-like polypeptides (ELPs) are artificial biopolymers containing repeats of the pentapeptide sequence Val-Pro-Gly-X-Gly, where X can be any amino acid except Pro (128). Human IL-10, Murine IL-4, Anti-HIV Type I antibody 2F5, Anti-foot and mouth disease virus single variable antibody fragment, Human Tissue Transglutaminase, etc. have been stably recovered by fusion with an ELP tag (118,128-130). Although these tags have been widely explored for fusion with different proteins, the fused tags can alter the folding of recombinant proteins and, in turn, their function. Apart from this, sometimes the tag can also interfere with the recovery of the product (131). So, the approaches related to fusion tags need to be optimized to reduce the disadvantages of these recombinant fused protein tags.

Similarly, a plant oleosin served as a 'carrier' for the synthesis of the leech anticoagulant protein, hirudin (variant 2), through the expression of the oleosin-hirudin fusion protein, which accumulated in seeds, constituting 1 % of the total seed protein. An *Arabidopsis* oleosin promoter mediated the expression and accurately localized to the oil body membrane. The recombinant hirudin protein underwent purification via anion exchange chromatography and reverse-phase chromatography and the approach offers a method for the large-scale synthesis and purification of recombinant proteins or polypeptides in plants (132). For example, elastin-like polypeptides (ELPs) and artificial biopolymers (133, 134) are fusion partners to increase plant recombinant protein accumulation. Interferon-gamma, human IL-10 and murine IL-4 (129), the full-size anti-human immunodeficiency virus type 1 antibody 2F5 (130) and anti-foot and mouth disease virus single variable antibody fragment (118) have been stably recovered by fusing them to ELPs tags. Fusion augments the accumulation rate, hydrolysis resistance and improved solubility, preventing protein aggregation and denaturation of proteins in plants at elevated protein concentrations (121). This helps to concentrate on protein bodies formed in the leaves and seeds of tobacco, shielding them from further adverse effects and enhancing overall protein production (128) (Fig.2).

Gene editing approaches

Genetic diversity is necessary to keep pace with natural evolution and adapt to all weather conditions (135). After the 1970s, the development of recombinant DNA technology (RDT), along with improved computational technologies and sequencing methods, brought significant strides in the biological sciences (136). It led to the development of transgenic crops (e.g., Bt Cotton), animals (Dolly) and several products (e.g., insulin, hCG) by insertion/deletion of desired genes with the help of restriction enzymes, vectors and ligase enzymes (137).

Recently, advanced genetic engineering technology has been developed to enable precise changes to be made in the genome of prokaryotes and eukaryotes. This technology has been termed gene editing (138). This became possible due to the development of sequence-specific, engineered endonucleases, including meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and type II clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) (139). The use of such nucleases is for the generation of double-stranded DNA breaks (DSBs) in a site-specific manner. This break is harnessed to modify targeted loci in the gene, as eukaryotic cells with induced double-strand breaks (DSBS) can be repaired either via the error-prone end-joining pathway or the error-free homology-directed repair (HDR) pathway (140). The CRISPR-Cas9 system, an RNA-directed DNA endonuclease adapted from the bacterial immune system, is one of the powerful gene editing tools. This tool was first used for plant gene editing in 2013 (141, 142). CRISPR/Cas9 technology involves two components, namely a single-strand guide RNA (sgRNA) and Cas9 (CRISPR-associated protein 9). The former component matches a desired target gene, whereas Cas9 is an endonuclease that makes double-strand breaks (DSBS). These two components' functions allow modification or gene editing (143). CRISPR-Cas9 has given hope to treat challenging genetic disorders caused by single-gene mutations such as cystic fibrosis (CF), Duchenne's muscular dystrophy (DMD) and haemoglobinopathies, or the potential for the treatment of diseases such as HIV (143) (Fig. 3).

Such great technology is not free from IP disputes. In a

long IP litigation dispute between the two prominent research groups, one is the Broad Institute led by Feng Zhang Broad Institute, Harvard University and the Massachusetts Institute of Technology and other is the other Charpentier and Doudna representing of the Max Planck Institute for Infection Biology, University of California, Berkeley (UCB) and the University of Vienna. Finally, the Broad group was granted the patent in 20114 to use CRISPRCas9 for eukaryotic cell editing methods (144). Since then, the battle has not yet been completely settled. The other group, led by Charpentier and Doudna, won the Nobel Prize in Chemistry for discovering the Cas-9 enzyme's role in CRISPR. The Indian patenting system is also influenced by ripples of CRISPR technology, as there is significant complexity in determining the patentability of CRISPR-related inventions. But the Indian Patent Office (IPO) has granted a patent to ERS Genomics, co-founded by Dr. Emmanuelle Charpentier, in May 2020 for "Methods and compositions for RNA-directed target DNA modification and for RNA-directed *modulation of transcription*" (145). Since then, many patent applications about CRISPR-based inventions, such as diagnostic kits and gene therapy, have been filed. Recently, India became the first country in the world to develop two genome-edited rice varieties (DRR Dhan 100 (Kamala), Pusa DST Rice 1) by ICAR institutions aimed to build climate-resilient, high-yield agriculture that can contribute to heralding the second green revolution in the country (146). Both varieties are developed using the Site Directed Nuclease 1 (SDN1) genome editing approach that created precise mutations without incorporating any foreign DNA and the resulting mutant line demonstrated superior yield performance, drought tolerance, high nitrogen-use efficiency (NUE) and maturity ~20 days earlier (around 130 days) than its parent variety (147), As these are created without adding or deleting any foreign gene so free from GM issues. These varieties are also exempted from the stringent biosafety regulations under Rules 7-11 of the Environment (Protection) Act, 1986 (147).

Biosafety, genetic engineering and molecular farming

Biosafety, legal framework at the international level

PMF is a biotechnology-supported advanced tool that offers perspectives to produce value-added products like novel recombinant proteins and diverse beneficial biochemical compounds for humans with therapeutic or consumptive value from genetically modified or engineered organisms (148). These products also value the pharmaceutical or industrial sectors as technology allows for large-scale production at minimal costs, easy raw material storage and less risk of contamination during processing from human or animal diseases (149). Countries worldwide have different legal frameworks for protecting genetically engineered organisms or products, including molecular pharming. However, concern for biosafety is incorporated uniformly and strictly in legal instruments conforming to international legal instruments.

European Union

European Union applies a very restrictive approach to dealing with GM-related products (150) about the deliberate release of GMOs into the environment by implementing Directive 2001/18/EC and Directive (EU) 2018/350(EC 2001) (151) or regulating pharmaceutical and therapeutic product for GM modified food and feed through the Regulation (EC) No 726/2004 under the auspices of the European Medicines Agency (EMA). Apart from that, Regulation (EC) No 1829/2003 governs GM food and feed,

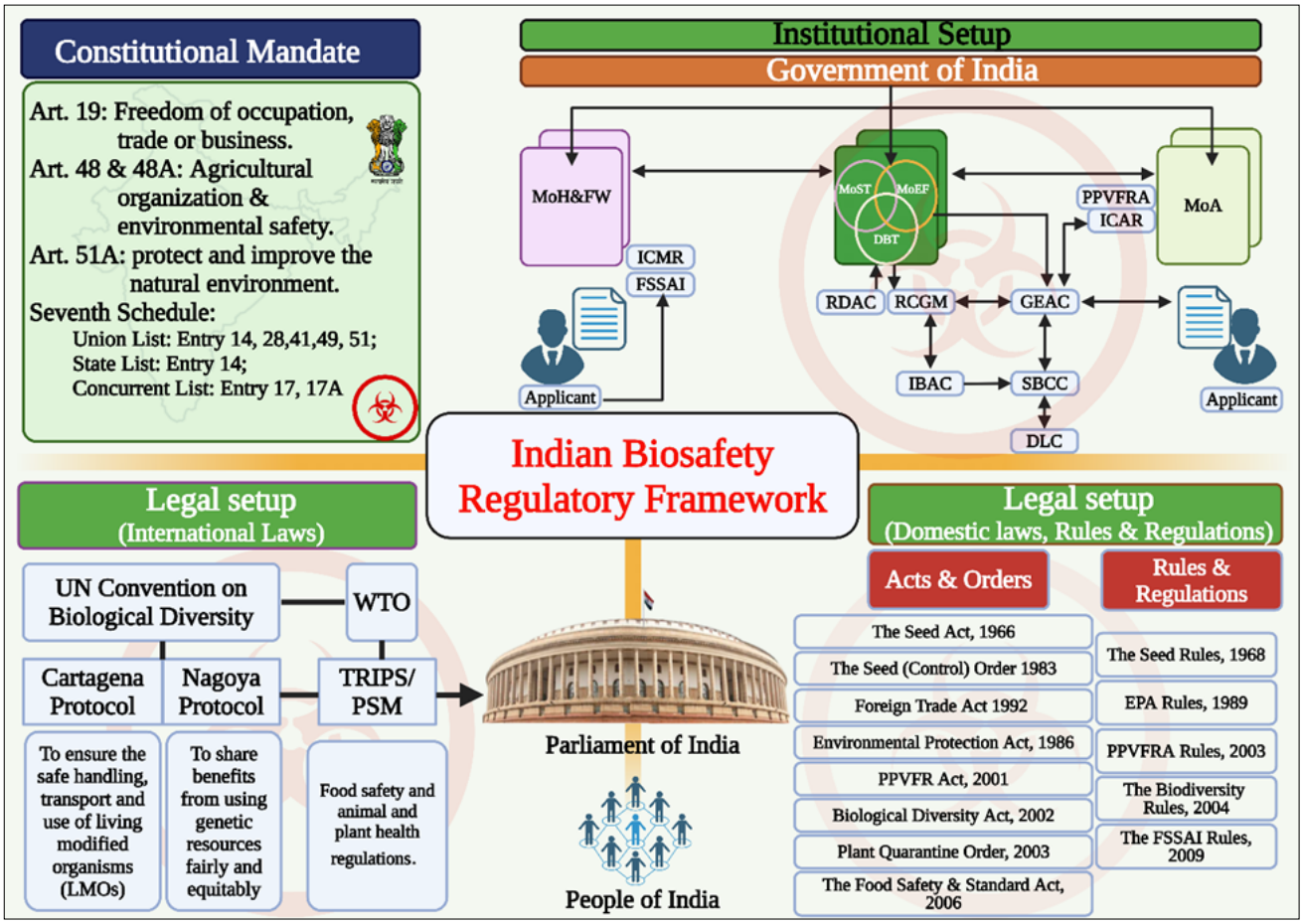

Fig. 3. Diagrammatic representation of bio-safety regulatory framework setup in India.

which must be adhered to when utilizing leftover biomass from GM agricultural plants as food or feed. The European Food Safety Authority (EFSA) plays a vital role in risk assessment, collaborating closely with member states. Directive 2009/41/EC specifically pertains to actions involving GM microorganisms (EC 2009) (152). These regulatory frameworks also cover the contained use of GM farming plants. The advent of application-based new genomic technologies (NGT), such as CRISPR/Cas, has simplified and accelerated GM development.

United States of America

Currently, genome-edited plants are regulated through the prohibition of the procedure or properties of the product, which laws are made by different governments to control to ensure biosafety and biosecurity concerns associated with plant genome editing. The USDA considers genome editing equivalent to conventional breeding, but Canada has applied scientific criteria to its domestic regulatory system. On the other hand, Argentina has established a functioning regulatory mechanism to approve genome-edited goods (153). The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) oversees genetically modified plant studies in the United States (154). The Food and Drug Administration (FDA) oversees the safety aspects of pharmaceutical products. These regulatory authorities also supervise the risk evaluation of genetically modified crops. The APHIS mandates that these GMOs adhere to a more stringent "permit" protocol, which includes particular confinement measures and procedures for compliance verification.

United Nations Convention on Biological Diversity

The United Nations Convention on Biological Diversity (CBD) is a specific international law that deals with biodiversity, environmental cum intellectual property rights-related aspects. It was adopted at the Earth Summit in Rio de Janeiro in 1992. The leaders agreed on a comprehensive strategy for sustainable development, recognizing biodiversity as an integral part. The Convention delineates three primary objectives: the preservation of biological variety, the sustainable utilization of its components and the equitable distribution of benefits derived from using genetic resources (155). India became a signatory to the Convention in December 1993 and ratified it in February 1994. India enacted the Biological Diversity Act 2002 (156) to fulfil this commitment. Before this Act, the Government of India regulated such activities by framing rules through an umbrella legislation known as the Environmental Protection Act 1986 (157).

Cartagena protocol

Cartagena Protocol (CP) is an international agreement within the ambit of the CBD to achieve its goals (158). The protocol is based on the "*precautionary principle*". It seeks to guarantee the secure management, transportation and use of LMOs derived from modern biotechnology that could negatively impact biological diversity, ecosystems and human health. The protocol derives its legal authority from Article 19 of the CBD. Member parties adopted the Protocol on January 29, 2000, but it came into force on September 11, 2003. Article 19 pertains to the management of biotechnology and the allocation of its benefits (158). Article 19(3) and (4) are dedicated to biosafety and lays down a protocol setting out appropriate procedure, including advance informed consent, safe transfer, handling and use of any living modified organisms (LMO) resulting from biotechnology-based activities that may have an adverse effect on the conservation and sustainable use of biodiversity (155). Article 19(4) also makes an obligation for each contracting party to ensure the available information from the natural or legal person providing the organism intended to be used as described under Article 19(3) of the Convention (158).

Nagoya protocol

Nagoya protocol (NP), made under the preamble and Article 5 of CBD that mandates member parties are legally obliged to follow rules to prevent bio-piracy by applying PIC and ensure benefit sharing, including financial benefits, to other Parties after legally accessing genetic resources (159). These protocols were further strengthened by adopting a supplementary protocol named the Nagoya-Kuala Lumpur supplementary protocol on 15 October 2010 by the fifth meeting of the Conference of the Parties (COPs) to the CBD. Nagoya Protocol is an ancillary protocol designed to enhance the CP on Biosafety by establishing international regulations and procedures for liability and remediation for biodiversity damage caused by LMOs. The protocol addresses administrative procedures and rules related to measures necessary for damage caused by LMOs that negatively impact biodiversity conservation and sustainable use while considering hazards to human health (159).

Biosafety and Legal Framework in India

Before the CBD, India ensured biosafety and biosecurity provisions as per constitutional and prevailing statutes. Indian Constitution provides that all the permitted or customary activities that don't harm living organisms or adversely impact the natural ecosystem are allowed or otherwise restricted and legislative bodies at the union and state levels are responsible for enacting relevant laws, orders, rules and regulations to ensure this (160). As per the Indian constitutional setup, three tiers of courts, starting from the lower court, high court and Supreme court of India, work to scrutinize, interpret and adjudicate the matter when any dispute arises or sometimes take *suo motu* cognizance when the matter seems large and in the public interest (161). Indian Constitution also provides the fundamental right to practice any profession or to carry on any occupation, trade or business under Article 19(1)(g) to all citizens with certain reasonable restrictions in the light of the sovereignty and integrity of India, the security of the State, public order, decency or morality (162). The Constitution also gives directives to the State to organize agriculture and animal husbandry, protect and improve the environment and safeguard forests and wildlife under articles 48 and 48A (163); all these aspects include and require biosafety vigilance. Similarly, the Constitution has laid out the duties of the citizens. However, it is not binding. It directs the citizens to protect and improve the natural environment, including forests, lakes, rivers and wildlife and to have compassion for living creatures under Article 51A(g) (164). This has been further strengthened by Article 21, which talks about the Right to life (164). The Supreme Court of India has held that the right to a clean environment is a fundamental right under the right to life in various judgments. Therefore, any activity that causes concern to the environment, including biosafety issues, may fall under the security (biosecurity) of the state and the environment and require regulation (164).The powers have also been vested in the union and state legislature by providing a list of subjects

divided into union, State and concurrent lists under the seventh schedule of the constitution. The key entries under the union list are 14, 28, 41, 49 and 51, whereas the State and the concurrent list contain entries 14, 17, 17A and 33 respectively, containing the direct or indirect implementation of biosafety (165).

India also made the Rules for the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms/ Genetically Engineered Organisms or Cells in 1989 (hereinafter 'Rules, 1989') under the Environment (Protection) Act, 1986 (EPA 1986). The Ministry of Environment, Forest and Climate Change (MoEF & CC) notified us of this rule (166). These rules are meant for all activities related to GMOs or cells and non-GE hazardous microorganisms and products to ensure safety from using GMOs and products thereof in research and application, to the uses and the environment that bring all under the scrutiny purview of biosafety (167). The Rules 1989 are applied along with Revised Guidelines for Research in Transgenic Plants & Guidelines for Toxicity and Allergenicity Evaluation of Transgenic Seeds, Plants and Plant parts-1998 (168).

As a party to the CBD, India has enacted a domestic law regulating biodiversity and ancillary matters, known as the Biological Diversity Act 2002 (BD Act), under a commitment made while adopting the Convention. It contains provisions for biodiversity conservation by *in-situ* and *ex-situ* measures under Section 37(1) of the BD Act, 2002(157). The Act also ensures the prevention of biopiracy by regulating the use of genetic resources, stipulating that permission must be sought before use by the NBA for commercial and research purposes and while making any IP application (169). The Act also ensures the database and records of biodiversity available at local places through listing in the People's Biodiversity Register (PBR) under the local control through biodiversity management committees (BMCs) across India (156).

To ensure biosafety and regulate the use of LMO or GM organisms, the multilevel institutional mechanism is working in India as follows (170):

- The Recombinant DNA Advisory Committee
- Institutional Biosafety Committee
- Research Committee for Genetic Manipulation (RCGM)
- Genetic Engineering Approval Committee (GEAC)

The Recombinant DNA Advisory Committee (RDAC) operates under the Department of Biotechnology (DBT), Government of India and is responsible for analysing biotechnology-related advancements nationally and internationally. It also advocates for proper regulatory measures for recombinant research and its applications in India as needed (171). Institutional Biosafety Committee (IBSC) works at the lowest level of execution, i.e., an institutional-level mechanism for biosafety (171) comprises an occupier or any individual, including a research institution, managing dangerous microorganisms or genetically altered organisms at the research and development (R & D) stage. The committee shall ensure biosafety measures that include taking notes, approving r-DNA work, ensuring adherence to r-DNA safety guidelines, preparing a guideline-based emergency plan, recommending to RCGM about category III risk or above experiments and seeking the Research Committee for Genetic Manipulation (RCGM) to inform the district-level committee (DLC), state biodiversity coordination committee (SBCC), as well as Genetic Engineering Approval Committee (GEAC) regarding the experiments as necessary and serves as a central point for interaction with regulatory authorities (171).

The Research Committee for Genetic Manipulation (RCGM) also works under the supervision of the DBT. To ensure environmental safety, it is mandated to develop guidelines/ manuals delineating procedures for the regulatory process concerning GMOs in research, utilization and applications within the industry. The committee evaluates all ongoing r-DNA projects classified as high-risk and controlled field experiments and permits the importation of GMOs/transgenes for research purposes. RCGM shall establish protocols that limit or forbid the manufacture, marketing, importation and utilization of genetically altered organisms or cells specified in the Schedule of Rules, 1989 (170).

The Genetic Engineering Approval Committee (GEAC) is the apex committee that regulates biosafety related to LMO/GM organisms and their products at the national level in India. The committee operates under the MoEFCC and has the power to sanction the utilization or approval of GMOs and their derivatives for commercial purposes, establish protocols for the restriction or prohibition of the production, sale, import and application of GMOs for both research and practical use under the EPA, permit large-scale production and environmental release of GMOs and their derivatives and empower agencies or individuals to enact punitive measures under the EPA (168).

On the other hand, SBCC is assigned the task of inspecting, investigating and imposing sanctions for breaches of rules/laws via the nodal department and the State Pollution Control Board (SPCB)/Directorate of Health & Medical Services (DHMS). The committee monitors GMO-related activities at the state level by periodically reviewing the safety and control measures in the various installations/institutions handling GM organisms/hazardous microorganisms (171).

The district-level committee oversees activities related to GMOs at the district level, akin to the SBCC. The DLC or any authorized individuals shall inspect the installation involved with GM organisms and hazardous microorganisms, create an information chart, identify hazards and risks associated with each installation and coordinate activities to address emergencies. The DLC also must develop an off-site emergency strategy for field experiments. The DLC consistently submits its report to the SBCC/ GEAC (170). Food Safety and Standards Authority of India (FSSAI) is an authority set up by the Ministry of Health and Family Welfare (MoH & FW), a dedicated authority under the FSS Act 2006. The authority has released a regulation known as FSSAI Rules 2011 that contains provisions for food product analysis, lifting a sample for testing microbiological parameters, biological emissions and sanitation and maintenance of establishment premises for biological agents.

India has also decided that gene-edited crops and foods will not be as tightly regulated as GMOs (172). The decision was taken in the light of a recommendation made by the Ministry that genome-edited products under the labels SDN1 and SDN2 “free from exogenous introduced DNA” be exempted from biosafety assessments located within Rules of the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms/ Genetically engineered Organisms or Cells Rules 1989” (173).

Recently, two varieties of rice (Kamala' and 'Pusa DST Rice 1) were developed using the Site Directed Nuclease 1 (SDN1) genome editing approach, released by relaxing Rules 7-11 of the Environment (Protection) Act, 1986 (174). India is not the only country to do so; the UK has also released a Statutory Instrument to conduct trial research on gene-edited plants. Other countries include Australia, Canada, Japan, Argentina, Brazil and the U.S., where simple gene-edited plants are not regulated as GMOs (175). Therefore, the decision not to regulate tightly might have been considered because several ethical issues or potential side effects of the gene editing technique are not yet known to us and countries are keeping it out of tight regulatory purview to harness its potential benefits. The gene editing technology can be further reclassified based on the availability of negative issues in future.

The PMF outputs are sometimes contained within the seeds, apart from fruits or other plant body parts. Hybrid GM seeds are also used as agricultural inputs to increase agricultural production. Consequently, ensuring the protection of farmers' and consumers' health and financial state by providing genetically pure and high-quality seeds is essential. The Government of India enacted the Seed Act, 1966 (176) and associated Seed Rules, 1968 (177) to ensure the marketing and equal distribution of the seeds. Additional subordinate legislation, such as the Seed (Control) Order 1983, was promulgated under the Essential Commodities Act of 1955 to keep further vigilance. These laws contain provisions for regulatory measures, seed classification, certification, restrictions on importing and exporting seeds, labelling, seed inspectors and penalties for violating these rules (178). Seeds exhibit properties such as dormancy, nutrient storage and seedling vigour that make them ideal for PMF applications, especially when many proteins are required. Transgenic plant seeds are used to generate a raw material for the extraction and isolation of proteins, polypeptides and non-proteinaceous compounds like antibodies and other immunoglobulins, which can be processed into valuable biopharmaceuticals that can be stored for a longer time due to their dormancy and low water content properties. Insulin, HGF, lysozyme and lactoferrin are examples of plant-derived molecules for which preclinical and clinical information is required to progress them through the research phase and into the regulatory pathway, ultimately leading to approval. To regulate such products in the agricultural and market sectors, a robust legal system is required (179).

The Government of India enacted the Seed Act, 1966 (176) and Seed Rules, 1968 (177) to ensure marketing and equal distribution of the seeds. For this purpose, a central committee is constituted, central and state seed laboratories are established for seed analysis and authorities are appointed. After consultation with the committee, the Act empowers the Central Government (CG) to notify the kinds or varieties of seeds that can be sold for agricultural purposes. The power of CG is also to lay down the minimum limits of germination and purity with respect to any seed of any notified kind or variety and/ or the mark or label to indicate that such seed conforms to the minimum limits of germination and purity specified under clause (a) and the particulars which mark or label may contain and can regulate on the grounds of seed identity, germination level, purity, mark or label or any other requirements prescribed. Additional subordinate legislation, such as the Seed (Control) Order 1983, was promulgated under the Essential Commodities Act (ECA) of 1955 to maintain further vigilance by requiring those dealing in seeds to be licensed. The license allows one to conduct the business of selling, exporting, or importing seeds anywhere, except under and by the terms and conditions of the license granted. This ensures the fair availability of seeds in general and in adverse conditions when public interest requires. To ensure this, the appropriate authority for enforcement and punishment provisions is incorporated.

The Indian patent system doesn't recognize LMOs under plant varieties for IPRs. The Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS), which is part of the Agreement that established the World Trade Organization (WTO) in 1995, mandates that member countries, including India, implement a system for plant variety protection via patents, *sui generis* legislation, or a combination of both under Article 27.3(b) (180). India chose to legislate a *sui generis* legislation to protect the plant varieties by enacting the Protection of Plant Variety & Farmers Rights (PPVFR) Act, 2001 and Rules made thereof in 2003. It makes provisions for registering new varieties based on novelty, distinctiveness, uniformity and stability (N-DUS). The Act provides an effective system for the registration of new plant varieties based on novelty, distinctiveness, uniformity and stability (N-DUS) and the farmers', plant breeders', communities and researchers' rights. In this way, the Act also helps conserve, improve and make available plant genetic resources (PGR) to develop new plant varieties and stimulate investment in research. Critical issues such as benefit sharing and the use of genetic material for new variety development have been addressed through establishing a national gene fund (NGF) and imposing penalty provisions for applying false denomination or false information about variety in the Act and Rules (181). PMF and the PPVFR Act are very important and complementary. New plant varieties having economic potential are recognized under the PPVFR Act. To ensure biosafety, the Act incorporates provisions under Sections 18 and 29. Section 18 includes the rider that an affidavit sworn by the applicant shall accompany every application presented for registration of a new variety under Section 14 that such variety does not contain any gene or gene sequence involving terminator technology; or include complete passport data of the parental lines from which the variety has been derived along with the geographical location in India from where the genetic material has been taken and all such information relating to the contribution, if any, of any farmer, village community, institution or organization in breeding, evolving or developing the variety; or contain a declaration that the genetic material or parental material acquired for breeding, evolving or developing the array has been lawfully acquired (181) .

Apart from these, biosafety concerns of human health, living organisms and the ecosystem is ensured by protection, prohibition and regulation on the import of agricultural articles into India through Section 3(1) of Plant Quarantine (Regulation of Import into India) Order, 2003 issued under the Destructive Insects and Pests Act (DIPA), 1914 by the Central Government. Section 3(1) lists the general conditions for importing plants, plant products, soil, etc., regulates permits for importing germplasm, transgenic or GMOs, live insects, microbial cultures and post-entry quarantine mechanisms (182).

The GM Approval Database (GMAD) is a feature of the

International Service for the Acquisition of Agri-biotech Applications (ISAAA). It consolidates all available information regarding biotech products (which can be molecular farming products) and GM crops approved for cultivation, importation for food and feed and commercialization. The database shows that 32 GM food crops have received approval in various countries (183).

The Genetic Engineering Appraisal Committee has sanctioned five genes/events of Bt cotton, one BN Bt cotton variety and one NHH Bt cotton hybrid. In India, cotton is the sole GM crop approved for commercial purposes (184) and 38 recombinant therapeutics have been approved for marketing in India till the writing of this paper (184). However, trials are being conducted on additional transgenic crops such as brinjal, chickpea, maize and tomato. Recently, the GEAC considered the release of GM mustard hybrid DMH-11 and Parental lines containing events developed using barnase, barnstar and bar genes developed by M/s. Centre for Genetic Manipulation of Crop Plants (CGMCP), University of Delhi, is advancing it toward full commercial cultivation (185) as in the 134th GEAC meeting held on March 21, 2018, for its environmental release (186). The committee resolved that the applicant must conduct a field demonstration of GM Mustard over 5 acres at 2-3 distinct sites to collect supplementary data on honeybees, other pollinators, honey production and soil microbial diversity to guarantee biosafety and associated considerations. The 147th meeting, convened on October 18, 2022, reviewed the submission and noted, “Based on the global examination of scientific evidence and the recommendations of relevant ministries, it appears improbable that the bar, barnase and barstar system will adversely affect honeybees and other pollinators.” Consequently, the committee posited that GEAC should contemplate the environmental release of GE mustard and undertake further assessment following ICAR rules for release and notification (187). If approved, GM mustard will be the second GM crop and first food crop after Bt Cotton to be allowed for cultivation in India and may open the path for other GM crops waiting for approval. However, there is no doubt that GM plants or products/gene-edited crops using CRISPR/Cas9 must undergo a stringent health and environmental risk assessment before they reach consumers or are released to be grown in nature (150,188).

The National Green Tribunal (NGT) is a green court established by the Government of India under the NGT Act, 2010, for adjudicating environmental matters and ancillary subjects, including GMOs. Biotechnology can potentially target diverse organisms and traits, creating novel GMOs and increasing the spatial and temporal scale is likely to occur (189). Therefore, there is a start of discussion on evolving laws on post-market environmental monitoring (PMEM) that can arise (190).

All these legal measures are enough to ensure the various dimensions of PMF, genetic engineering and biosafety in India. Still, the Indian legal system dynamically adapts to protect the environment and human health (Fig. 4). Countries have adopted different legal and institutional measures to minimize the risks associated with GM crops or their products (Table 1).

Opportunities and Challenges

PMF-derived biopharmaceuticals or other valuable products satisfy the criteria of safety, efficacy, performance and utility, as do other production techniques' products. They will be considered to yield economically beneficial products to countries like India, where mass demand is placed. Currently, many products are ready for commercial use but are pending approval from regulating agencies such as GEAC or the MoH & FW, or require further tests and evaluation. These agencies must apply stringent standards and precautionary principles in international laws about biodiversity, IPR and trade regimes to apply measures for unbiased evaluation to ensure minimum risk after approval. Apart from human or animal health, another potential risk is considered an environmental concern, as anticipated when transgenic pollen is transported to weeds or allied crops (200-202). Expressing hazardous pharmaceutical proteins in non-target plants resulting from such outcrosses may generate a negative public perception, which must be addressed to reduce the risk and associated apprehension. If these challenges are tackled successfully, releasing such transgenic crops will become easy and provide opportunities to harness the vast hidden potential of this technology. Despite the considerable advancement of molecular farming in recent years, many challenges must be addressed. A primary constraint in the broader utilization of plant hosts to produce important recombinant proteins is the poor yield of complete recombinant proteins. One of the major causes of such low yield is the proteolytic degradation of recombinant proteins, which poses a significant challenge and requires developing a novel strategy to overcome this issue (203).

Conclusion

After the Second World War, countries across the globe became independent and started developing with time. However, many countries, including India, are still passing through the transition stage and a large population is living in poverty with low and uncertain income. Such sections of society need value-added food and good health care at affordable prices. Here, PMF can be very important in such countries to serve the needs of people by making available food, feed and medicinal products like edible vaccines generated through bio-factories based products such as fruits, seeds and vegetables that can be helpful to prevent malnutrition and diseases in adults and children’s and minimize infant mortality rate. These vaccines or plant products are cheaper and can be stored in normal conditions, providing additional benefits. Other products like enzymes, antibodies, proteins and metabolites also have great potential for human day-to-day use and industrial use. Therefore, there is great hope that the future of molecular farming will help humanity overcome several problems by making cheaper and readily available bioproducts. Health safety and environmental biosecurity are ensured by adopting international laws, enacting legislation as per constitutional mandate, laying down rules and institutional support to execute and monitor these rules, which are well-created in India. India follows and fulfils international laws under CBD, Cartagena Protocol and Nagayoa Protocol to ensure global commitments. Still, releasing GMOs and their products is under more vigorous scrutiny from public and institutional frameworks. It requires more scientific awareness to increase the public perception of its pros and cons that can enhance better acceptability in public.

Acknowledgements

The authors thank their respective Institutional Heads for

Table 1. Summary of legislation/articles/rules concerned with PMF and biosafety

S. No.	Article/Provision; Name of Legislation and Year	Description	Reference
		INTERNATIONAL LEVEL	
1.	Article 15; Convention on Biological Diversity; 1992 Article 6, Nagoya Protocol	Access to genetic resources.	155, 159
2.	Article 19; Convention on Biological Diversity; 1992 Article 4; Cartagena Protocol; 2003	Handling of biotechnology and distribution of its benefits. The transboundary movement, transit, handling and use of all living modified organisms that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health.	155 191
3.	Article 27; WTO-TRIPS, 1995	Patentable Subject Matter;	180
		National Level	
1.	Article 19(1)(g) The Constitution of India, 1950	All citizens with certain reasonable restrictions in the light of the sovereignty and integrity of India, the security of the State, public order, decency, or morality; The Constitution of India, 1950,	162
2.	Article 21, The Constitution of India, 1950	Right to life: The Constitution of India, 1950, includes the Right to a decent environment, including pollution-free water and air, as well as protection against hazardous industries and health hazards. (Andhra Pradesh Pollution Control Board v. M.V. Nayudu Subhash Kumar v. State of Bihar Susetha v. State of Tamil Nadu Municipal Council, Ratlam v. Vardichan)	164 192 193 194 195
3.	Articles 48, The Constitution of India, 1950	The State shall endeavour to organise agriculture and animal husbandry on modern and *scientific lines* and shall, in particular, take steps for preserving and improving the breeds and prohibiting the slaughter, of cows and calves and other milch and draught cattle. The Constitution of India, 1950	163
4.	Articles 48A, The Constitution of India, 1950 Article 51A(g), The Constitution of India, 1950	The State shall endeavour to protect and improve the environment and to safeguard the forests and wildlife of the country; To protect and improve the natural environment, including forests, lakes, rivers and wildlife and to have compassion for living creatures;	163 164
5.	Entry no. 14, 28, 41, 49 and 51 of the Union List, The Constitution of India, 1950.	Entering into treaties and agreements with foreign countries and implementing treaties, agreements and conventions with foreign countries (Entry 14); Port quarantine, including hospitals connected therewith; seamen's and marine hospitals (Entry 28); Trade and commerce with foreign countries; import and export across customs frontiers; definition of customs frontiers (Entry 41); Patents, inventions and designs; copyright; trade-marks and merchandise marks (Entry 49); Establishment of standards of quality for goods to be exported out of India or transported from one State to another (Entry 51).	165
6.	Entry no. 6, 14 and 17A of State list; The Constitution of India, 1950.	Public health and sanitation; hospitals and dispensaries (Entry 6); Agriculture, including agricultural education and research, protection against pests and prevention of plant diseases (Entry 14);	165
6.	Entry no. 17, 17A, 17B and 33 of the Concurrent list; The Constitution of India, 1950.	Prevention of cruelty to animals (Entry 17); Forests (Entry 17A); Protection of wild animals and birds (Entry 17B) 33. Trade and commerce in and the production, supply and distribution (a) the products of any industry where the control of such industry by the Union is declared by Parliament by law to be expedient in the public interest and imported goods of the same kind as such products; (b) foodstuffs, including edible oilseeds and oils; (c) cattle fodder, including oilcakes and other concentrates; (d) raw cotton, whether ginned or unginned and cotton seed; and (e) raw jute.]	165
7.	Section 3, The Environment (Protection) Act, 1986; Rule 2, 7-11, 20; The Rules for the Manufacture, Use, Import, Export and Storage of Hazardous Microorganisms/Genetically Engineered Organisms or Cells, 1989.	The power of the Central Government to take measures to protect and improve the environment. Rule 2, 7-11, 20 dealing with application, approval and prohibitions, etc., production, deliberate or unintentional release, permission and approval for certain substances, permission and approval for foodstuffs and exemption	157
8.	Section 3-7; The Biological Diversity Act 2003 and related rules	Regulation of access to biological diversity.	156
9.	Section 13, 22; Food Safety and Standards Act, 2006 and Rules 2011.	Scientific Panels: Genetically modified foods, organic foods, functional foods, proprietary foods, etc.	196
	Section 7; The Seed Act, 1966 and Seed Rules, 1968	Regulation of the sale of seeds of notified kinds or varieties.	176, 177
	Section 3; The Essential Commodities Act of 1955.	Powers to control production, supply, distribution, etc., of essential commodities.	197
	Section 3-10; The Seed (Control) Order 1983.	Dealer in seeds to be licensed.	178

Sections 15 and 29 ; The Protection of Plant Variety & Farmers Rights (PPVFR) Act, 2001 and Rules made thereof in 2003	Registrable varieties. Exclusion of certain varieties.	181
Sections 3 and 4; The Indian Patent Act 1970 and rules.	The section deals with “Inventions not patentable”.	198
Section 3; The Destructive Insects and Pests Act, 1914	Power of the Central Government to regulate or prohibit the import of articles likely to infect.	199
Section 3-11 The Plant Quarantine (Regulation of Import into India) Order, 2003	General conditions for import; Special conditions of Import, Post-entry Quarantine;	182

providing the necessary support and facilities to complete this MS. Sheetal is thankful to UGC for the Junior Research Fellowship (F. No. 16-6 (DEC.2017)/2018(NET/CSIR).

Authors' contributions

AKM conceptualized, drafted, wrote, created figures and edited the manuscript; YPS, S, BK, KG, SB, TB and GK participated in writing and editing. DS supervised, composed and edited the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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