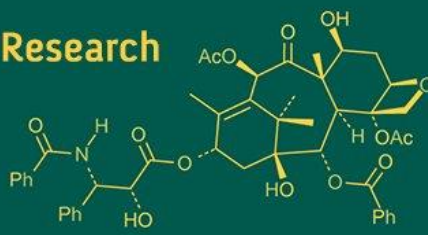


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**Saikat Jana**  
Department of Horticulture,  
School of Agriculture, Lovely  
Professional University,  
Punjab, India

**Manzoor A Khanday**  
Department of Statistics,  
Lovely Professional  
University, Punjab, India

**Kajal Singh**  
Department of Horticulture,  
School of Agriculture, Lovely  
Professional University,  
Punjab, India

## Biotechnological applications of gene cloning in vegetable crop improvement

Saikat Jana, Manzoor A Khanday and Kajal Singh

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### Abstract

Gene cloning is a crucial fashion in agrarian biotechnology that has greatly contributed to advancements in vegetable wisdom. It enables precise manipulation and revision of specific genes associated with desirable traits analogous as pest and complaint resistance, forbearance to abiotic stresses and bettered nutritional quality. This technology plays a pivotal part in developing superior vegetable kinds able of meeting the adding demands of sustainable husbandry. The process involves appropriate a target DNA scrap into a suitable vector, which is also introduced into a host organism for replication. *Agrobacterium tumefaciens*- derived modified vectors, especially binary vector systems that improve transformation efficiency are frequently used to transfer genes in plant systems. DNA manipulation involves essential enzymes including polymerases, DNA ligases and restriction endonucleases. Because of its quick growth and simplicity of handling, *Escherichia coli* is frequently utilized as a host organism, whereas yeast and plant cells are used for eukaryotic gene expression. Antibiotic resistance indicators, blue-white screening, PCR and DNA sequencing are among the selection and screening methods used to verify the successful integration of recombinant DNA.

**Keywords:** Gene cloning, recombinant DNA, vectors, transformation, genetic modification

### 1. Introduction

Gene cloning has developed into a powerful tool in present-day agricultural biotechnology that offers exact techniques for improving vegetable crops. As important providers of vitamins, minerals, antioxidants and dietary fiber, vegetables are essential to human nutrition. Though, the global vegetable production is gradually challenged by factors such as climate change, declining natural resources and the growing demand for food due to rapid population increase. According to the global estimates, an agricultural production must increase significantly to meet the food requirements of a population expected to exceed 9 billion by 2050 (Alexandratos & Bruinsma, 2012) [3]. At the same time, malnutrition and micronutrient deficiencies continue to affect the millions of people at the worldwide and highlighting the urgent need for nutrient-rich crop varieties. Conventional breeding methods, although it was successful in the olden, are often needs a lot of time and limited by genetic variability and species barriers. On another side, gene cloning focused in effective system for separating, altering, and transferring particular genes linked to characteristics. Using this system, DNA fractions are fitted into applicable vectors, which are latterly fitted into the host systems for expression and replication. Gene cloning has greatly improved our knowledge of plant biology and trait regulation by making it possible to investigate and modify gene function (Zhong *et al.*, 2023) [75]. Gene cloning has been extensively used in vegetable crops to boost forbearance to abiotic stresses including saltiness and failure, improvement resistance to pests and conditions and extend shelf life and nutritive quality. Also, it forms the base for slice- edge technologies like CRISPR- Cas systems, recombinant DNA technology and RNA hindrance (RNAi). These developments have created new openings for crop improvement and perfection parentage (Rajput *et al.*, 2021) [45]. The thing of this paper is to give a thorough understanding of gene cloning styles and how they're used in vegetable wisdom. It discusses the difficulties and eventuality of this snappily developing discipline while pressing the most current developments, useful operations and new trends. The study highlights the eventuality of gene cloning to support sustainable husbandry, increased food security and bettered nutritive issues through this discussion.

**Corresponding Author:**  
**Saikat Jana**  
Department of Horticulture,  
School of Agriculture, Lovely  
Professional University,  
Punjab, India

## 2. An overview of gene cloning

Gene cloning involves the combination of a target gene into an extra chromosomal DNA molecule known as a vector, which serves as a carrier for the genetic manipulation (Brown, 2025) <sup>[15]</sup>. After that, this vector is put into a host cell generally a bacterium where it helps the fitted gene spread and replicate. The vector and the bacterial genome duplicate formerly inside the host, producing multitudinous identical clones of the target gene and the host cell.

The recombinant DNA molecule is copied and passed on to the daughter cells as the bacterial cells divide. A group of genetically identical cells known as a clone and it's created by repeated cycles of cell division. The gene has been successfully reproduced since each cell in this clone has one or further clones of the recombinant DNA. Wide reaching modification of particular DNA sequences is made possible by this method, which is vital for further inheritable exploration and use (Brown, 2016) <sup>[14]</sup>.

## 3. Steps which are involve for these applications

### Identification of the gene of interest

Choosing and segregating a particular DNA scrap or target locus that encodes the asked protein is the first stage in the gene cloning process. Direct gene insulation from a genomic library in factory systems is constantly delicate for two primary reasons. First of all, because factory genomes are generally huge and complicated, chancing the gene of interest requires screening a large number of duplicates. Second, shops have genes with introns since they're eukaryotic organisms, which can make downstream gene expression exploration more gruelling (Anusha *et al.*, 2025) <sup>[6]</sup>. Reciprocal DNA (cDNA) libraries are constantly employed to overcome these constraints. The first step in creating a cDNA library is to prize total RNA from tissue or cells that are laboriously making the target protein. Messenger RNA (mRNA), which represents the expressed genes, is uprooted from this total RNA. The rear transcriptase enzyme is latterly used to transfigure the mRNA into reciprocal DNA (cDNA). Because it has no introns, this cDNA offers a better template for cloning and expression exploration (Brown *et al.*, 2018) <sup>[12]</sup>. Following medication, the cDNA library is screened to find duplicates that contain the asked gene. Approach like nucleic acid hybridization and immunological discovery approaches can do this. Also, a quick and effective system for amplifying and segregating the target gene sequence is the polymerase chain response (PCR) (Wong, 2018) <sup>[68]</sup>.

### Selection of an appropriate vector for cloning

A gene of interest is carried and introduced to produce recombinant DNA by cloning vectors, which are technical DNA motes that can replicate singly within a host organism. A vector needs to have a many crucial rates in order to work well. These include a comparatively bitsy size that makes running, separation and inheritable manipulation easier and the capacity to replicate effectively within the host cell, performing in numerous clones of the fitted gene. In order to identify successfully converted cells, a good vector should also have a selectable marker, most frequently an antibiotic resistance gene. Alike vector to replicate and maintain itself properly, compatibility with host organism is also essential (Lodge *et al.*, 2007) <sup>[35]</sup>. Plasmids and bacteriophages are the

most commonly applied vectors in recombinant DNA technology considering of their high replication usefulness and ability to carry foreign DNA (Verma *et al.*, 2022) <sup>[66]</sup>. Particularly, plasmids utilize special sequences called the origins of reduplication to control how numerous clones they've inside bacterial cells. For example, the plasmid pUC18 has a high duplicate number that can reach up to 100 clones per cell, while the plasmid pBR322 commonly occurs in moderate duplicate figures, extend from around 12 to 20 clones per bacterial cell (Khan, 1996) <sup>[32]</sup>.

The intended operation has a major part in choosing the right vector. When a significant volume of gene product is demanded, high duplicate number plasmids like pUC18 are favored, still medium duplicate number plasmids like pBR322 are more appropriate when it's critical to lessen metabolic stress on the host cell (Lodge *et al.*, 2007) <sup>[35]</sup>.

As a natural vector for introducing foreign DNA into plant cells, the Ti plasmid has been used considerably. It comes from the soil bacteria *Agrobacterium tumefaciens*, which infects plants and causes crown bitterness complaint, which causes growths that act excrescences (Hooykaas, 2023) <sup>[29]</sup>. By means of acidity (vir) genes, a particular section of the plasmid known as transfer DNA (T- DNA), which is around 20 kb in size, gets fitted into the plant genome during the infection process. Following combination, T- DNA manages the expression of genes that multiply lump development and unrestricted cell proliferation (Gelvin, 2017) <sup>[25]</sup>.

The natural Ti plasmid has a number of downsides while being a successful gene transfer medium. For illustration, its huge size (about 150 - 200 kb) makes it challenging to isolate, manipulate and introduce into host cells under laboratory conditions and the presence of excrescence converting genes makes it inappropriate for direct use in inheritable engineering. Scientists have created modified Ti plasmid variants for securer and further effective use in order to overcome these limitations. The double vector system, which isolates the acidity genes from the T- DNA region, one out of the most popular styles.

In this approach, the vir genes are kept on a helper plasmid inside *Agrobacterium*, while the gene of interest and selectable marker genes are carried on a lower cloning vector. This configuration improves metamorphosis performance and makes manipulation easier. To construct a feasible fantastic vector, another tactic called the cointegration approach combines factors of the Ti plasmid with a bacterial plasmid, like pBR322. Remarkable advancement in vector design inrecent times have increased utilize in vegetable crops. Strong promoters, multitudinous cloning spots and journalist genes have been incorporated into contemporary binary vectors like pCambia and pBIN19 to facilitate effective gene transfer and selection in crops including tomato, brinjal, pepper and cucurbits (Baskar *et al.*, 2019) <sup>[9]</sup>.

Likewise, advanced cloning styles like the Gateway and Golden Gate systems enable the modular construction of several genes, making it easier to heap characteristics linked to nutrition and resistance. New developments in synthetic biology are also helping to overcome numerous of the drawbacks of conventional Ti plasmid- grounded systems by creating acclimatized vectors that are lower, more stable and compatible with a wider range of hosts.

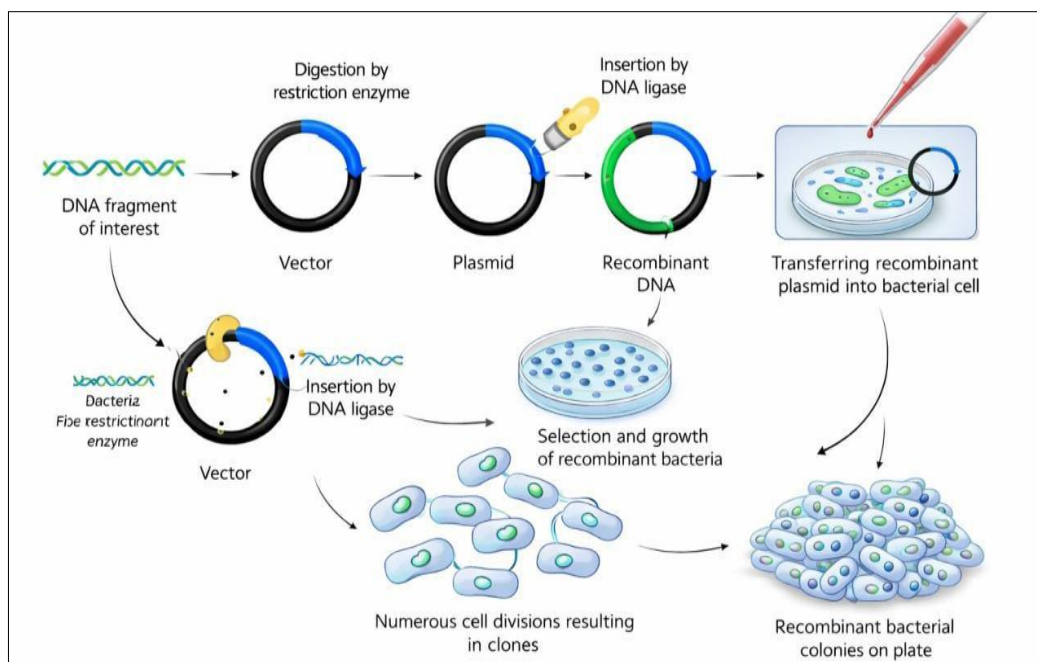


**Fig 1:** Diagrammatic illustration of the gene cloning process

### Essential enzymes required for cloning procedures

A collection of technical enzymes that allow for exact manipulation of DNA molecules are essential to gene cloning. Among these, restriction endonucleases are essential because they stick DNA at particular nucleotide sequences called restriction sites. The phosphodiester links in the DNA backbone are broken by these enzymes, producing fragments with either blunt or solid (sticky) ends. Digestion with a restriction enzyme constantly results in multitudinous different DNA fragments because a specific restriction site may appear multiple times inside a DNA patch. Once the DNA sequence is known, all possible fractionalization spots for constantly used enzymes can be set up by creating a restriction map, a procedure that's presently effectively completed exercising bioinformatics tools (Wong, 2018) [68]. Type II restriction enzymes are especially important for copying because they give elegant gene insertion by cutting DNA at exact and prognosticated places (Brown *et al.*, 2016) [14]. The enzyme that joined DNA fragments following DNA division is called DNA ligase. Bacteriophage T4 is the source of the most generally used ligase, which efficiently forms phosphodiester bonds to bridge the spaces between DNA segments. Because restriction enzymes and DNA ligase work together, scientists can cut and assemble DNA molecules in a controlled way (Lodge *et al.*, 2007) [35].

Additionally, DNA polymerases are important for cloning processes. For DNA combine and modification, Taq polymerase and DNA-dependent DNA polymerases such as those from T4 and T7 bacteriophages and *Escherichia coli* are constantly used. Furthermore, the synthesis of reciprocal DNA (cDNA) from RNA templates requires reverse transcriptase, an RNA-dependent DNA polymerase (Hubscher *et al.*, 2010) [30].

Alkaline phosphatase is another pivotal cloning enzyme that's used in vector manufacture (Berger *et al.*, 1987) [10]. This enzyme stops the vector from self-ligating or re-circularizing without the preface of foreign DNA by removing phosphate groups from the 5' ends of DNA. This stage guarantees that the target gene is successfully

combined into the vector and improves ligation effectiveness.

### Identifying the Host Organism for Cloning

Due to its multiple applied advantages, *Escherichia coli* is considered the most ideal host organism for gene cloning. One of its main advantages is how facily it can be managed and cultivated applying conventional laboratory forms, which simplifies and improves experimental operations (Zhang, 2000) [73]. Further, a variety of genetically modified host strains and cloning vectors have been created to modify gene expression and better cloning results. Effective experiment design and troubleshooting are supported by a thorough understanding of the physiology and genetic of *E. coli*. This system capacity to quickly generate recombinant proteins is another expressive benefit. Significant Quantities of protein, frequently in the milligram range, can be generated in a matter of weeks after eukaryotic cDNA duplicates are expressed. Its connection for artificial operations is farther influenced in well-established large-scale fermentation techniques. In addition, *E. coli* is a cost-effective option for biotechnology research and manufacturing, due to its relatively low cultivation costs and capability to produce large volumes of recombinant proteins (Salem-Bekhit *et al.*, 2013) [50]. BL21 (DE3), DB 3.1, JM110 and Stbl2 are frequently used strains for molecular cloning (Bertero *et al.*, 2017) [11].

However, *E. coli* is a prokaryotic organism and it lacks the cellular machinery demanded for post-translational changes, which are necessary for numerous eukaryotic proteins to serve properly. Alternative host systems are used to get around this restriction. Because yeast cells can carry out specific post-translational changes, they're extensively utilized to examine eukaryotic gene expression. Plant-derived recombinant proteins are also produced using plant cell cultures, similar as hairy root and suspension cultures. Other technical eukaryotic systems, including the protozoan *Trypanosoma brucei* and the nematode *Caenorhabditis elegans* are also employed in particular exploration settings where it's necessary to examine further intricate cellular procedures (Dey & Chakrabarti, 2018)

**Table 1:** Most commonly vectors those are used in these applications

| Vector                                | Insert size  | Purpose                              | Example              |
|---------------------------------------|--------------|--------------------------------------|----------------------|
| Plasmid                               | 10-20kb      | Protein expression, DNA manipulation | pBR322, pUC18, pCMV  |
| Cosmid                                | ~45 kb       | Genomic libraries                    | pHM, pLAFR1, pJB8    |
| Bacterial Artificial Chromosome (BAC) | 130-150 kb   | Genomic libraries                    | pBeloBAC11, pBACe3.6 |
| Yeast Artificial Chromosome (YAC)     | 1000-2000 kb | Genomic libraries                    | pYAC4                |
| Phage ( $\lambda$ , insertion)        | ~10 kb       | cDNA libraries                       | $\lambda$ gt11       |
| Phage ( $\lambda$ , replacement)      | ~23 kb       | Genomic libraries                    | EMBL4                |

(Anusha *et al.*, 2025) <sup>[6]</sup>

### Transformation

The process of introducing foreign DNA into living cells is known as transformation. The performing recombinant DNA construct must to be moved into an applicable host cell for replication and expression once a desired gene has been introduced into a vector. The type of host organism and the particular pretensions of the cloning experiment determine which metamorphosis fashion is best (Rajpathak *et al.*, 2022) <sup>[44]</sup>.

*Agrobacterium tumefaciens*- intermediated metamorphosis is one of the most considerably used techniques in plant biotechnology (Singh & Prasad, 2016) <sup>[61]</sup>. This strategy can be implemented using a variety of methods, similar as seedling infection, protoplast co-cultivation and explant inoculation. Because it constantly produces stable gene integration with comparatively low duplication numbers, it's especially effective in vegetable crops including tomato, brinjal, pepper and cucurbits (Rahman *et al.*, 2024) <sup>[42]</sup>.

Chemical treatment, normally with calcium chloride (CaCl<sub>2</sub>) or related salts, can enable cells in bacterial systems to absorb exogenous DNA (Li *et al.*, 2010) <sup>[34]</sup>. These treated cells, appertained to as competent cells, are able of effectively absorbing DNA from their environment.

Another popular system is electroporation, which involves subjugating cells to a short electrical pulse that shortly damages the cell membrane, allowing DNA molecules to enter the cell (Ho & Mittal, 1996) <sup>[28]</sup>. However, other ways like biolistic (gene gun) transformation are preferred in some plant species, similar as onion and garlic, that are less open to *Agrobacterium*- based procedures (Eady, 2002) <sup>[22]</sup>. To enrich delicacy and effectiveness, fresh and creative transformation techniques have been created in recent times. These include protoplast electroporation methods, CRISPR-associated Ribonucleoprotein (RNP) delivery and gene delivery systems based on nanotechnology (Alghuthaymi *et al.*, 2021) <sup>[4]</sup>. These developments present viable ways to get over species-specific constraints and multiply transformation success rates in a variety of vegetable crops.

### Identification of the transformants for cloning

Transformants are cells that have successfully combined foreign DNA into their inheritable composition. These cells are recognized using a range of screening and selection techniques, which is a vital step in the gene cloning methods. Reporter genes that generate observable or measurable signals, blue-white screening that divides recombinant colonies according to colour differences and antibiotic selection in which only cells containing the recombinant DNA survive are common methods. Molecular methods similar colony PCR, individual restriction digestion and DNA sequencing are used to further confirm the presence and correctness of the inserted gene. In certain

situations, positive selection vectors are also employed to improve the successfully of detecting real transformants.

### Techniques those are used for cloning

Whether the study's pretensions are to dissect gene function, produce recombinant proteins, or perform inheritable revision, the choice of a respectable cloning fashion is heavily influenced by those pretensions. There are several methods accessible, each applicable for particular experimental conditions and results. Below is a discussion of some of the most popular methods.

#### Isolation of the DNA techniques

Segregating and purifying plasmid DNA from converted *Escherichia coli* cells is a pivotal stage in gene cloning. The miniprep fashion, which enables the quick birth of plasmid DNA from a bitsy volume of bacterial culture-generally about 1 ml of a late- overgrown culture is one of the most popular approaches for this purpose (Akhtar, 2021) <sup>[2]</sup>. Alkaline lysis is the first step in the procedure, which involves treating cells with a result of Sodium Hydroxide (NaOH) and Sodium Dodecyl Sulphate (SDS). Cellular factors are released into the result as a result of this treatment's dislocation of the bacterial cell wall and membrane. Potassium acetate is used to neutralize the admixture after lysis, which aids in the separation of plasmid DNA from proteins, lipids and chromosomal DNA. Incipiently, the plasmid DNA is extracted by precipitation, generally using 95 cold ethanol. This produces pure DNA that can be used in subsequent processes like cloning, sequencing, or transformation.

#### Gel electrophoresis techniques

A popular method for sorting natural molecules including DNA, RNA and proteins according to their size and electrical charge is gel electrophoresis (Rana & Joshi, 2023) <sup>[46]</sup>. The samples are situated in a gel matrix and the molecules move through the gel whenever electric field is applied. Due to differences in size and charge, molecules move at varying speeds; smaller molecules often travel further and faster than larger ones. One type of gel electrophoresis that can be used to separate proteins and nucleic acids with high resolution is Polyacrylamide Gel Electrophoresis (PAGE) (Maizel, 1971) <sup>[37]</sup>. This technique makes use of a polyacrylamide gel matrix, which enables the detection of even the smallest differences in molecule size.

#### Southern transfer techniques

The process of transferring DNA fragments that have been separated on an agarose gel onto a solid membrane, generally composed of nitrocellulose or nylon, is called Southern transfer, frequently referred to as Southern blotting. The DNA fragments are exposed to a labeled probe

that is reciprocal to the target sequence after they have been fixed on the membrane. By hybridizing with DNA fragment the probe enables the detection and analysis of sequence (Brown, 1993) <sup>[13]</sup>.

### Colony-based PCR analysis

Bacterial colonies can be quickly and effectively screened for presence of certain DNA sequence using colony-based PCR. Using this system, the individual colonies are taken straight from agar plate and utilized as PCR response DNA template. The presence of intended recombinant DNA insert in colony is indicated by modification of target area. This method is useful tool for gene cloning examinations since it enables rapid detection of positive clones without demand for previous DNA extraction (Azevedo *et al.*, 2017) <sup>[7]</sup>.

### Modern enzyme-based and modular cloning systems

Modern cloning strategies have evolved to include advanced enzyme based and modular assembly technique that allow precise and effective establishment of recombinant DNA molecules (Ta *et al.*, 2025) <sup>[64]</sup>. Among these, Golden Gate cloning and Gibson assembly are extensively used for ideal DNA assembly. These methods rely on fragments with overlapping ends that joined through coordinated activity of enzymes similar as exonucleases, DNA polymerases and DNA ligases, enabling accurate and nonstop assembly of multiple DNA segments in single response. Another important approach is 3A BioBrick Assembly method, which is designed for high- throughput construction of inheritable elements. This technique utilizes standardized BioBrick part and antibiotic selection system, allowing effective assembly of DNA fragments without need for gel purification step. Similar advanced system significantly improved speed, flexibility and reliability of gene cloning workflows (Shetty *et al.*, 2011) <sup>[58]</sup>.

### Recombination-based and ligase-independent techniques

Recombination based and ligase independent cloning methods offer efficient alternatives to traditional ligation dependent approaches. These techniques often rely on the activity of enzymes such as T4 DNA polymerase to generate extended single stranded overhangs, which enable vector and insert DNA fragments to anneal without direct use of DNA ligase. This simplifies cloning process and makes it particularly suitable for high-throughput applications (Ijaz & Haq, 2019) <sup>[31]</sup>. Another one method is SLICE (Seamless Ligation Cloning Extract), which utilizes cell extracts from *Escherichia coli* containing natural recombination machinery to join DNA fragments with short homologous regions in a seamless manner. Other approach, TEDA (T5 exonuclease- dependent assembly) employs T5 exonuclease to create single stranded overhangs that facilitate efficient and accurate assembly of DNA fragments (Dong *et al.*, 2021) <sup>[21]</sup>.

In addition to this advanced method several supporting technique are commonly used in cloning workflow. These include DNA sequencing for validation, Polymerase Chain Reaction (PCR) for amplification site directed mutagenesis for introducing specific genetic changes and immunological as well as non-radioactive detection method for analysis and confirmation.

### Applications of gene cloning in the vegetable crops

RNA Interference (RNAi), CRISPR - Cas system recombinant DNA technology, gene therapy, synthetic biology and ultramodern molecular individual tools are just

a many of the advanced biotechnological approaches that support gene cloning. Gene cloning serves as pivotal platform for these technologies by making it possible to isolate and amplify particular genes (Brown, 2025) <sup>[15]</sup>. Gene cloning has several uses in vegetable science, especially in enhancing crop quality and performance. It is a key for raising output, building disease, pest resistance, boosting tolerance to environmental stress and boosting the nutritive content of vegetables. All effects considered, gene cloning makes a substantial contribution to the development of high-quality sustainable vegetable agriculture.

### Gene cloning for disease resistance

Gene cloning plays a vital part in resistance breeding by enabling accurate identification, isolation and transfer of resistance genes into susceptible vegetable crops. Through cloning approaches specific genes responsible for resistance can be obtained from plant varieties that naturally exhibit tolerance to particular pathogens (Young, 2017) <sup>[71]</sup>. Common strategies used for this purpose include map-based cloning and transposon tagging.

Map- based cloning is a widely used approach to identify genes associated with specific traits by locating inheritable markers positioned near target locus within the genome (Zhang, 2024) <sup>[72]</sup>. The process begins with identification of markers nearly linked to trait of interest, followed by narrowing down genomic region through genetic and physical mapping. Potential candidate genes are also isolated, cloned and functionally validated. This approach has been successfully applied in identifying genes responsible for disease resistance. For example, in Brassica oleracea map- based cloning was used to identify a candidate gene (Bra012688) associated with resistance to Fusarium yellows, following fine mapping of *FocBo1* locus using segregating populations (Shimizu *et al.*, 2015) <sup>[59]</sup>. Another important method is transposon tagging, which utilizes mobile inheritable elements that fit into genome and produce mutations. By tracking insertion sites and relating them with phenotypic changes, researchers can identify and study genes responsible for specific traits (Brutnell, 2002) <sup>[16]</sup>. This technique has been applied in tomato (*Solanum lycopersicum*) to clone and characterize resistance genes against tomato mosaic virus, including the durable Tm- 2<sup>2</sup> resistance gene (Mahfouze *et al.*, 2022) <sup>[36]</sup>.

Gene cloning also facilitates addition and transfer of resistance genes into different crop varieties, enabling protection against similar pathogens across species. For case, RNA interference (RNAi)- based strategies have been developed using cloning techniques to produce virus-resistant plants. For example, successfully generated okra shops resistant to Begomovirus using TA cloning and *Agrobacterium*- mediated transformation. TA cloning is a simple and effective method that exploits the property of Taq polymerase to add a single adenine nucleotide at the 3' ends of PCR products, allowing their direct ligation into vectors with reciprocal thymine overhangs (Green & Sambrook, 2021) <sup>[26]</sup>.

Molecular cloning has also been extensively used to characterize resistance genes in colorful vegetable crops. For example, the NBS- LRR gene *SacMi*, identified in a wild brinjal species (*Solanum aculeatissimum*), confers resistance to the root- knot nematode *Meloidogyne incognita* (Zhou *et al.*, 2018) <sup>[76]</sup>. Also, full- length cDNA of *BcTuR3* gene was isolated from Chinese cabbage using rapid-fire

amplification of cDNA ends (RACE) and cloned for farther analysis. This gene showed rapid-fire expression in response to infection by Turnip mosaic virus, indicating its role in plant defense.

Overall, gene cloning has become a necessary tool for understanding and enhancing disease resistance in vegetable crops, enabling development of improved varieties with durable and broad-spectrum protection.

### Enhancing nutritional quality through gene cloning

The nutritional value and post-harvest traits of vegetable crops have been greatly enhanced using gene cloning and advanced molecular techniques including RNA interference (RNAi) and CRISPR/Cas systems. By regulating the ripening process RNAi technology successfully increased the shelf life of tomatoes. The tomato short life cycle, ease of genetic alteration and established propagation techniques make it perfect model organism for researching fruit development (Gupta *et al.*, 2013) [27].

One of the key targets in ripening control is enzyme Poly Galacturonase (PG), which is responsible for the breakdown of pectin, which is important structural component of plant cell wall. Under normal conditions, the PG gene is transcribed into mRNA and translated into the functional enzyme, leading fruit softening. However, the introduction of antisense RNA can bind to mRNA and inhibit its translation, thereby reducing enzyme production. This results in firmer fruits with improved shelf life and better suitability for long-distance transport (Sheehy, 1988) [56]. Similarly, modification in N-glycan processing have been shown to influence fruit softening. Transgenic tomato lines with suppressed expression of  $\beta$ -hexosaminidase ( $\beta$ -Hex) and  $\alpha$ -mannosidase ( $\alpha$ -Man) genes exhibited significantly increased firmness-up to two to two-and-a-half times and extended shelf life of nearly 30 days (Meli *et al.*, 2010) [38]. RNAi has also been applied to alter metabolic pathways, such as suppression of chalcone synthase gene, which plays a role in flavonoid biosynthesis and has been linked to the development of seedless tomato varieties (Schijlen *et al.*, 2007) [51]. For the enhance of the nutritional value of vegetables have included the manipulation of genes involved in photomorphogenesis, such as DET1, to improve fruit quality. In potato, RNAi-mediated silencing of the Beta-Carotene Hydroxylase (BCH) gene has led to increased accumulation of beneficial carotenoids like beta-carotene and lutein (Van Eck *et al.*, 2007) [65]. Additionally, CRISPR/Cas9 technology has been used to target genes such as SLO1, resulting in delayed ripening in tomato (Yang *et al.*, 2017) [69]. Gene cloning has also contributed to addressing quality-related issues such as enzymatic browning. For instance, polyphenol oxidases, which cause discoloration in crops like eggplant upon cutting, can be targeted to improve visual and nutritional quality (Ebrahimi *et al.*, 2023) [23]. Moreover, advances in vegetable biotechnology have drawn inspiration from successful biofortification efforts in other crops, such as Golden Rice and folate-enriched banana (Paine *et al.*, 2005) [40].

Several studies highlight the practical success of these approaches in vegetables. Examples include the development of tomatoes enriched with provitamin A and folate through metabolic engineering, as well as spinach lines with enhanced iron and zinc content achieved via targeted gene overexpression (De la Garza *et al.*, 2004; Garg *et al.*, 2018) [17, 24]. Collectively, these advancements

demonstrate that gene cloning not only improves shelf life and texture but also enables precise nutritional enhancement, offering promising strategies to combat micronutrient deficiencies and improve overall food quality.

### Gene cloning for pest and herbicide resistance

Gene cloning has been widely applied to introduce the desirable genes into plant genomes, leading to the development of genetically modified crops with enhanced resistance to pests and herbicides. This approach plays a significant role in crop protection while supporting sustainable agricultural practices (Kumar *et al.*, 2020) [33]. Genes conferring insect resistance are sourced from a variety of organisms, including microorganisms, plants and animals and have been successfully identified, cloned and incorporated into many economically important crops (Sharma *et al.*, 2004) [54].

Among microbial sources, *Bacillus thuringiensis* (Bt), a soil bacterium, is one of the most extensively utilized. It produces crystal (Cry) proteins encoded by cry genes, which are toxic to specific insect larvae. Upon ingestion, these proteins disrupt the insect gut, leading to paralysis and eventual death. Similarly, Vegetative Insecticidal Proteins (VIP), also produced by BT, act by damaging the midgut lining of insects through osmotic lysis. Different categories of VIP proteins show specificity against various insect groups, such as coleopteran and lepidopteran pests (Adang, 2018) [1]. A well-known example of the successful application of cloning in pest resistance is Bt brinjal, where the *cry1Ac* gene was introduced to control fruit and shoot borer infestations (Pal *et al.*, 2009) [41]. Since its commercialization in Bangladesh in 2013, Bt brinjal has significantly reduced insecticide usage by more than 80% while improving farmer income and reducing exposure to harmful chemicals (Shelton *et al.*, 2019) [57]. Similar strategies using Cry and Vip genes are being explored in other vegetable crops such as tomato and cabbage, demonstrating the practical potential of cloning-based technologies (Syed *et al.*, 2020) [63].

In addition to microbial genes, plant-derived insect resistance genes also contribute to pest management. These include Protease Inhibitors (PI) and lectin genes. Protease inhibitors interfere with the digestive enzymes of insects, reducing nutrient absorption and leading to impaired growth and survival. As a result, insects exhibit reduced development and eventually die due to metabolic disruption (Divekar *et al.*, 2023) [20]. Several genes from both microbial and plant sources have been successfully utilized in cloning studies. Examples of microbial genes include *cry1Ab*, *cry1Ac*, *cry6A* and *cry1Aa*, while plant-derived genes include Tomato Proteinase Inhibitor-II, Tobacco Anionic Peroxidase and Cowpea Trypsin Inhibitor (CpTI). These genes have been introduced into crops like tomato and brinjal to confer resistance against major insect pests such as *Plutella xylostella* (Schuler *et al.*, 1998) [52].

Overall, gene cloning has provided an effective and environmentally friendly approach for developing pest-resistant vegetable crops, reducing dependence on chemical pesticides and enhancing agricultural sustainability.

### Gene cloning for stress tolerance

Crop growth and productivity are significantly affected by various abiotic stresses, including drought, high temperature, flooding, salinity and cold conditions. These

environmental stresses trigger a series of physiological and biochemical changes in plants, often resulting in damage to cellular structures and metabolic processes (Singh *et al.*, 2020) <sup>[60]</sup>. Gene cloning has emerged as a powerful approach to enhance stress tolerance in vegetable crops by introducing and overexpressing genes associated with stress adaptation. For example, the *mtlD* gene, which encodes mannitol-1-phosphate dehydrogenase, has been introduced into tomato plants under the control of the CaMV 35S promoter. This modification improved the plant's ability to withstand various abiotic stresses (Rai *et al.*, 2022) <sup>[43]</sup>. Similarly, the salt-responsive gene *MdCIPK6L*, isolated from apple, has been transferred into tomato, where its overexpression conferred enhanced tolerance to drought, salinity and low-temperature conditions (Deng *et al.*, 2013) <sup>[18]</sup>.

In potato, the introduction of the *CodA* gene from *Arthrobacter globiformis*, which is involved in the synthesis of glycine betaine, has been shown to improve drought tolerance (You *et al.*, 2019) <sup>[70]</sup>. Likewise, the overexpression of the *Sobadh* gene from spinach in sweet potato resulted in increased resistance to salinity, cold stress and oxidative damage. This improvement was associated with better maintenance of cellular integrity, enhanced photosynthetic efficiency, reduced accumulation of Reactive Oxygen Species (ROS) and activation of detoxification mechanisms (Zhao *et al.*, 2025) <sup>[74]</sup>. Further advancements include the introduction of the *AVP1* gene from *Arabidopsis thaliana* into bottle gourd, which improved salt tolerance and helped maintain higher relative water content under stress conditions (Wang *et al.*, 2023) <sup>[67]</sup>. Recent developments in Next-Generation Sequencing (NGS) and high-throughput screening have accelerated the identification of stress-responsive genes in vegetable crops, particularly for traits related to drought, salinity and temperature resilience (Roy *et al.*, 2024) <sup>[48]</sup>.

Moreover, modern strategies such as multi-gene cloning and gene stacking are being employed to combine multiple stress tolerance traits within a single plant, enhancing its ability to withstand diverse environmental challenges. These approaches have shown promising results in crops like tomato, sweet potato and bottle gourd (Shailani *et al.*, 2021) <sup>[53]</sup>. Compared to cereals and fruit crops, vegetables offer distinct advantages for genetic improvement due to their shorter life cycles, relatively simple transformation systems and increasing demand for nutritional quality, making them ideal candidates for rapid advancement through gene cloning technologies (Rommens, 2007) <sup>[48]</sup>.

### Regulatory, ethical and intellectual property considerations

Beyond technological advancements, the application of gene cloning in vegetable crops is closely influenced by regulatory policies, ethical concerns and public acceptance. Regulatory systems vary significantly across regions. For example, the United States generally adopts a product-based approach, focusing on the characteristics of the final product, whereas the European Union follows a process-based framework, which evaluates the methods used in genetic modification. This often leads to more stringent regulations and longer approval processes for Genetically Modified (GM) crops in the EU (Sprink *et al.*, 2016) <sup>[62]</sup>.

Ethical considerations surrounding gene cloning primarily relate to food safety, environmental risks and potential

effects on biodiversity. Concerns about unintended ecological consequences and long-term impacts continue to shape public discourse. Consumer acceptance also remains a critical factor in the adoption of genetically modified vegetables. While the introduction of Bt brinjal in Bangladesh has demonstrated clear benefits, including reduced pesticide use and improved farmer livelihoods, similar technologies face resistance in other regions due to public perception, lack of awareness and labeling controversies (Shelton *et al.*, 2019) <sup>[57]</sup>.

Intellectual Property Rights (IPR) represent another important challenge in the field of gene cloning. Many key technologies, such as CRISPR/Cas systems and advanced cloning vectors are protected by patents, which can restrict access for researchers and plant breeders, particularly in developing countries. These limitations may hinder the development and dissemination of improved crop varieties that are accessible to small-scale farmers (Ravi *et al.*, 2025) <sup>[47]</sup>.

Therefore, it is essential to strike a balance between encouraging innovation and ensuring equitable access to these technologies. Future research and policy efforts should not only focus on scientific progress but also emphasize regulatory harmonization, ethical responsibility, public engagement and reforms in intellectual property frameworks. Addressing these aspects will help ensure that gene cloning technologies in vegetables are not only scientifically effective but also socially acceptable and widely accessible.

### Limitations and challenges in gene cloning of vegetable crops

Although gene cloning has contributed significantly to the improvement of vegetable crops, several technical, biological and ecological challenges still limit its widespread application. One of the major constraints is the low transformation efficiency observed in certain recalcitrant species, such as onion, garlic and some legumes, which makes the process less reliable and difficult to scale (Nivya & Shah, 2023) <sup>[39]</sup>. Another important limitation is the influence of genetic background on transgene performance. A gene that expresses effectively in one cultivar may not show the same level of expression or stability in another, leading to inconsistent results across different varieties (Bandopadhyay *et al.*, 2010) <sup>[8]</sup>. From an ecological standpoint, the possibility of gene flow from transgenic crops to wild relatives or non-modified cultivars raises concerns about biosafety, particularly in regions that serve as centres of crop diversity (Ammann *et al.*, 2012) <sup>[5]</sup>. In addition, unintended effects such as off-target mutations, metabolic imbalances, or pleiotropic responses may negatively impact plant growth, yield or quality.

Another challenge lies in translating laboratory success into field performance. Environmental variability often influences gene expression and interactions between genes and environmental factors can affect the stability and effectiveness of the introduced traits. Beyond scientific and technical issues, factors such as public perception, environmental sustainability and regulatory complexities continue to influence the adoption of gene cloning technologies in agriculture.

Overall, these challenges highlight the need for further research focused on improving transformation efficiency, developing genotype-independent systems, strengthening

biosafety evaluation and promoting transparent communication with stakeholders to ensure responsible and effective use of gene cloning in vegetable crops.

### Conclusion and Future prospects

Advancements in gene cloning have greatly enhanced our understanding of key biological processes in vegetable crops, including resistance to pests and herbicides, tolerance to abiotic stresses and the regulation of fruit ripening. These developments have provided a strong foundation for precise and targeted crop improvement. Looking ahead, research is increasingly focusing not only on stress tolerance but also on traits that directly benefit farmers, consumers and market demands. One important area of progress is the improvement of post-harvest shelf life. Genes involved in ethylene biosynthesis and signalling pathways are being manipulated to delay ripening. For example, tomato lines developed through antisense suppression of polygalacturonase or through CRISPR-based gene editing have shown promising results in reducing post-harvest losses. In addition, efforts are being made to enhance flavour and aroma by modifying metabolic pathways responsible for volatile compound production in crops such as tomato and pepper, thereby improving consumer acceptability.

Nutritional enhancement is another major focus, with biofortification strategies aimed at increasing the content of essential nutrients. Examples include tomato varieties enriched with pro- vitamin A and folate, as well as spinach and lettuce lines with improved iron and zinc levels that are currently under evaluation. Field-level successes further demonstrate the practical value of gene cloning. For example, Bt brinjal carrying the *cry1Ac* gene has already been commercialized in Bangladesh and is being evaluated in other regions for its effectiveness in controlling fruit and shoot borer. Similarly, cloned resistance genes are being tested in pepper against viral diseases, while molecular approaches are being applied in cucumber and squash to enhance virus tolerance. The integration of gene cloning with advanced tools such as genome editing and translational research is expected to drive the development of next-generation vegetable crops. These crops are likely to combine high yield, improved stress tolerance, extended shelf life, enhanced nutritional quality and better sensory attributes. Such innovations have the potential to reduce production costs, improve consumer health and satisfaction and minimize environmental impact. Ultimately, gene cloning will play a crucial role in building a more resilient and sustainable agricultural system.

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