

WEL COME





An
assignment
on

CLEAN GENE TECHNOLOGY

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M.Sc. (Agri)

Reg. no.: 04-AGRMA-01399-2015

Course Code:

Submitted to-

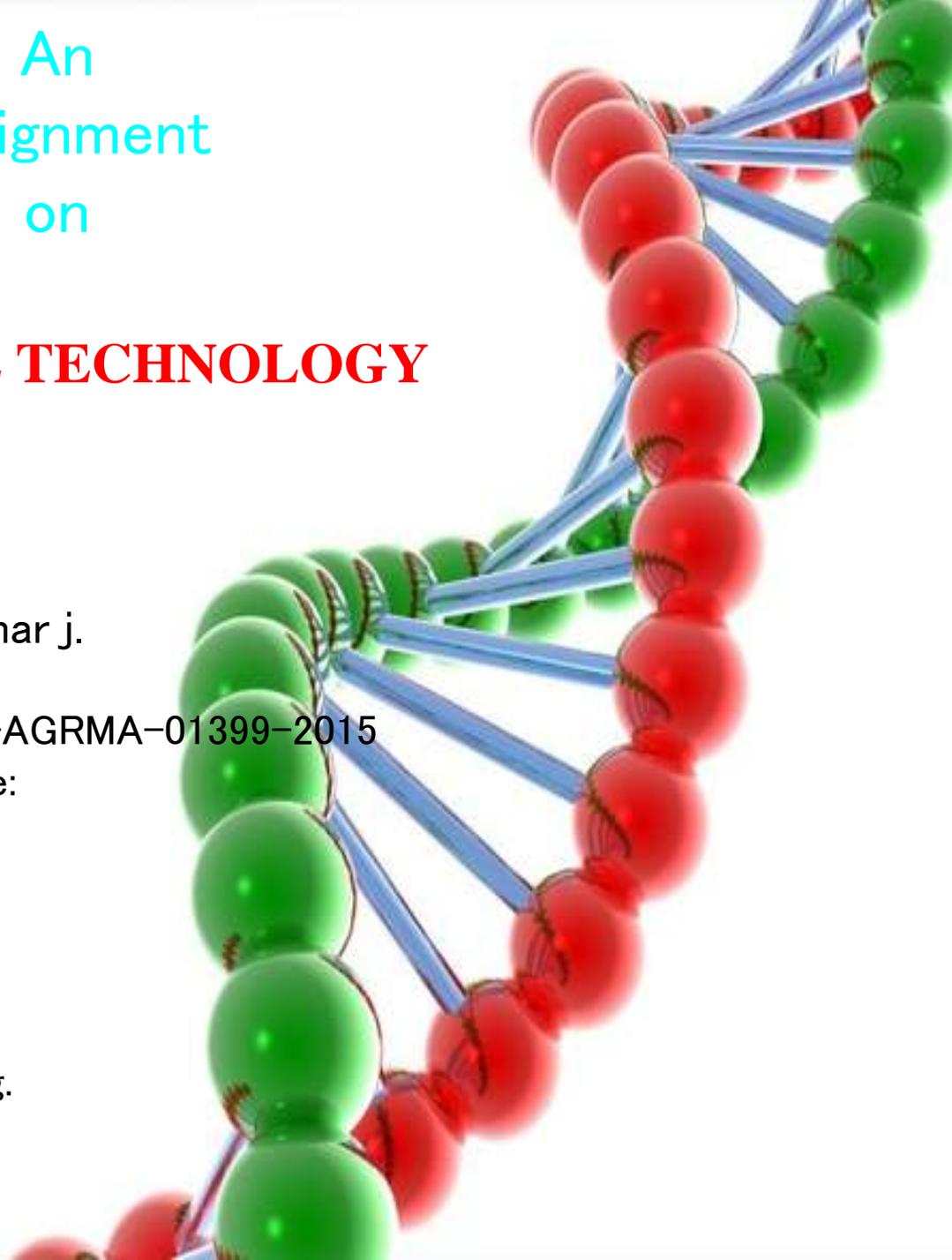
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INTRODUCTION



☞ **The process of developing transgenic plants without the presence of selectable markers (or) by use of more acceptable marker genes is regarded as Clean Gene Technology.**

Need of Clean Gene Technology



- ❧ The products of some marker genes may be toxic or allergic.
- ❧ The antibiotic resistance might be transferred to pathogenic microorganisms in the soil.
- ❧ There is a possibility of creation of superweeds that are resistant to normally used herbicides

Methods to Produce Marker Free Transgenic Plants



1. Co-transformation:

- ❧ Co-transformation is a method for production of markerfree transformants based on Agrobacterium- or biolistics mediated transformation in which a SMG and gene of interest are on separate constructs.
- ❧ SMGs can subsequently be removed from the plant genome during segregation and recombination that occurs during sexual reproduction by selecting on the transgene of interest and not the SMG in progeny.

Three approaches are used for co-transformation



- ❧ Introduction of two TDNAs, in separate *Agrobacterium* strains or Biolistics introduction of two plasmids in the same tissue.
- ❧ (ii) Introduction of two T-DNAs carried by different replicons within the same *Agrobacterium* strain.
- ❧ (iii) Introduction of two T-DNAs located on the same replicon within an *Agrobacterium*.

Disadvantages



- ❧ It cant be used for vegetatively propagated plants.
- ❧ These procedures not only require fertile plants, but are also very time consuming.
- ❧ Integration of an SMG and the transgene of interest on separate loci are required.

a) Using Plant DNA:

- Recent studies have shown that plants have T-DNA border like sequences in rice and Arabidopsis and these might be used in transformation. Because this so called Plant DNA (P-DNA).
- It lacks any Open Reading Frames(ORF) and contains a high A/T content, it is likely the footprint of ancient Agrobacterium-mediated natural transformation events via horizontal gene transfer.
- It has been demonstrated that plant-derived P-DNA fragments can be used to replace the universally employed Agrobacterium T-DNA for transformation.
- Co-transformation of the desired transgene into P-DNA is capable of producing marker-free transgenic plants.
- Inserting the bacterial *ipt* cytokinin expression cassette into the backbone of P-DNA vector enabled an increase in the frequency of backbone-free transgenic plant in the recovered population.

2. Site-Specific Recombination-Mediated Marker Deletion

- ✧ In temperate bacteriophages, there is a second type of recombination called Site-specific recombination, which takes place only between defined excision sites in the phage and in the bacterial chromosome.
- ✧ Positions of the site-specific recombination in the bacterial and phage DNA are called the bacterial and phage attachment sites, respectively.
- ✧ Each attachment site consists of three segments.
- ✧ The central segment has the conserved nucleotide sequence that sites the recombination event. A phage protein, an integrase, catalyzes the site-specific recombination events, which lead to physical exchange of DNA.

- ✧ Excision requires the phage enzyme integrase plus an additional phage protein called excisionase.
- ✧ There are three well-described site-specific recombination systems that might be useful for the production of marker-free transgenic plants:
- ✧ Cre/loxP system from bacteriophage P1, where the Cre enzyme recognizes its specific target sites.

Cre/loxP recombination system

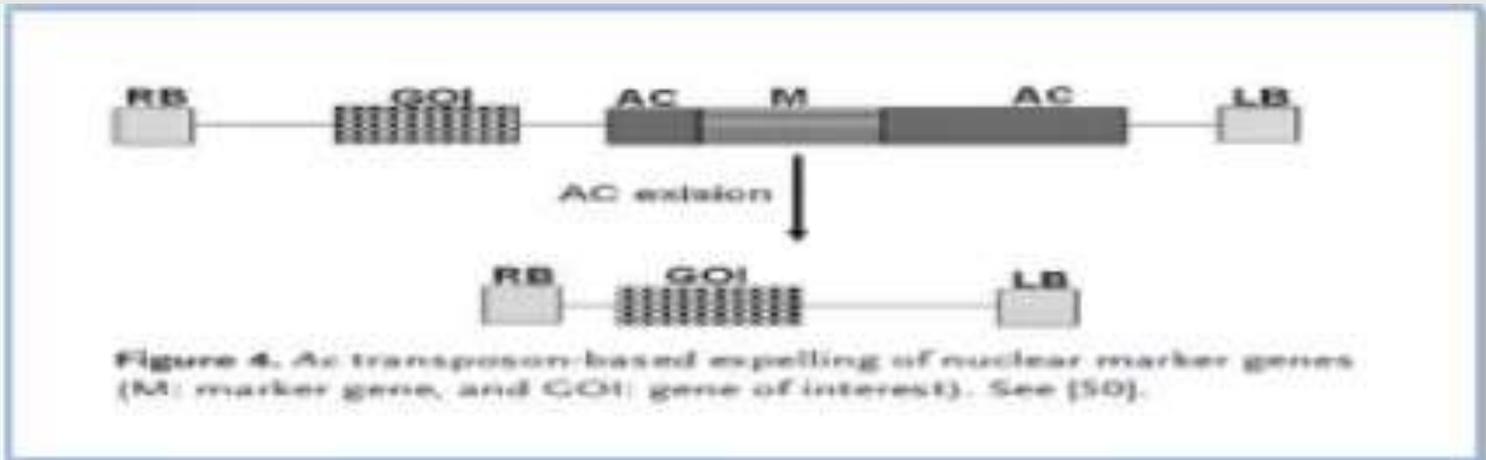


- ❧ In these systems, elimination of SMG would require recombinase expression in transgenic plants.
- ❧ The recombinase gene cassette can be introduced into transformed plants that contain the SMG between two recognition sites.
- ❧ Alternatively, a transgenic plant of interest can be crossed with a plant that expresses a recombinase gene.
- ❧ After segregation, marker-free transgenic progeny plants can be identified.
- ❧ A tightly controlled site-specific recombination system was recently employed in an efficient marker gene removal in tobacco pollen.

3. Transposon based marker methods



- ✧ In the 1940s, Barbara McClintock made an astonishing discovery. She detected two factors of DNA transposition in maize:
- ✧ a).Ds (disassociation) element that was located at a chromosome break site.
- ✧ b).Unlinked genetic factor (Ac) that was required to activate the breakage of chromosome
- ✧ Transposons are DNA sequences between hundreds to thousands of bases long. They code at least one protein, which enables them to replicate.
- ✧ The most widely studied transposon is the P element from the fruitfly (*Drosophila melanogaster*).



Steps:

- (i) Insertion of the marker gene onto a transposon, a segment of DNA that “hops” around in the plant’s genome;
- (ii) co-transformation with gene of interest
- (iii) Segregation of the marker gene.

Disadvantages

- (i) Different species have variable rates of transposition efficiency.
- (ii) This method requires labor and time costs for crossing transgenic plants and the selection of the progeny.
- (iii) There is low efficiency of marker-free transgenic plant generation, because of the tendency of transposable elements to reinsert elsewhere in the genome.
- (iv) Imprecise excision.
- (v) Generation of mutations because of insertion and excision cycles.
- (vi) Genomic instability of transgenic plants because the continuous presence of heterologous transposons decreases efficiency.

4. Intrachromosomal recombination system



- ❧ Recombination sites are engineered into the plant, but no recombinase is expressed. The attachment site from Phage origin is denoted *POP'* or *attP*, & the attachment site from *Bacterial* origin is denoted *BOB'* or *attB*.
- ❧ Intrachromosomal recombination in plants is obtained by insertion of SMG between two direct repeats of *attP* that facilitates spontaneous excision. Base composition of the *attP* site sequence is *A + T* rich, which is conjectured to play a recombination-stimulating role.
- ❧ Possibly, the formation of a recombination hot spot is caused via the induction of double-strand breaks (DSBs), but may also reduce the stability of transgene sequences later on.

The potential advantages

- ❧ (i) Expression of a heterologous recombinase and sexual reproduction are not necessary;
- ❧ (ii) There is a one step selection procedure for transgenic calli (lengthy propagation two-step time as above might increase the risk of somaclonal variation);
- ❧ (iii) It utilizes a natural nuclear recombination systems present in plants; (iv) the frequency of intrachromosomal recombination between two homologous sequences in plants might be increased by stimulation of repair systems; and
- ❧ (v) The efficiency of homologous recombination is directly correlated with the size of the homologous regions.

5. Removal of chloroplast marker genes



- ✧ Mitochondria and chloroplasts have independent genomes in plants that have been the target (especially chloroplasts) of genetic transformation. Biosafety might be facilitated by maternal inheritance, which is the case in most plant species, in which transgenes in plastids would not be disseminated via pollen.
- ✧ Chloroplast transformation vectors are designed with homologous flanking sequences on either side of the transgene..
- ✧ After recombination, co-integrates are inherently unstable because of direct repeats. Therefore, subsequent loop out recombination events create either the stable integration of a transgene of interest or loss of the integrated vector, which then yields a wild-type plastome.

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