AGRI MAGAZINE
(International E-Magazine for Agricultural Articles)
Volume: 01, Issue: 05 (December, 2024)
Available online at http://www.agrimagazine.in

Gene Stacking: Innovations in Modern Crop Improvement

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One kind of gene cloning is called "gene stacking," which is the act of integrating two or more desired genes into a single plant. Stacking is the term used to describe the combined features that emerge from this process. A biotech stack, sometimes known as a "stack," is a genetically modified crop variety that possesses stacked features. Roundup-ready cotton, which generates the EPSPS enzyme that confers resistance to the herbicide glyphosate, and bollgard cotton, which expresses the Bt toxin cry1ab, were crossed to create the first stack of cotton to receive regulatory certification in 1995.

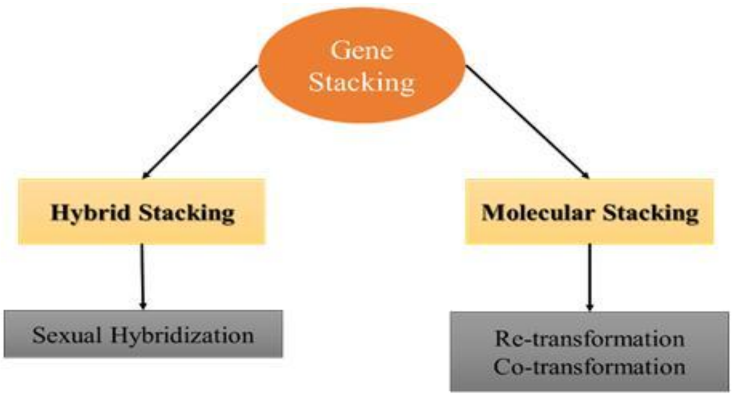
Need for Gene Stacking

- Stacks provide a more comprehensive agronomic improvement that enables farmers to satisfy their demands in challenging farming environments.
- Gene stacking enhances and streamlines pest management for biotech crops, as evidenced by multiple insect resistance based on Bt gene technology.
- Biotech stacks are designed to have better chances of overcoming the many issues in the field, such as insect pests, diseases, weeds, and environmental stresses, so that farmers can increase their productivity.

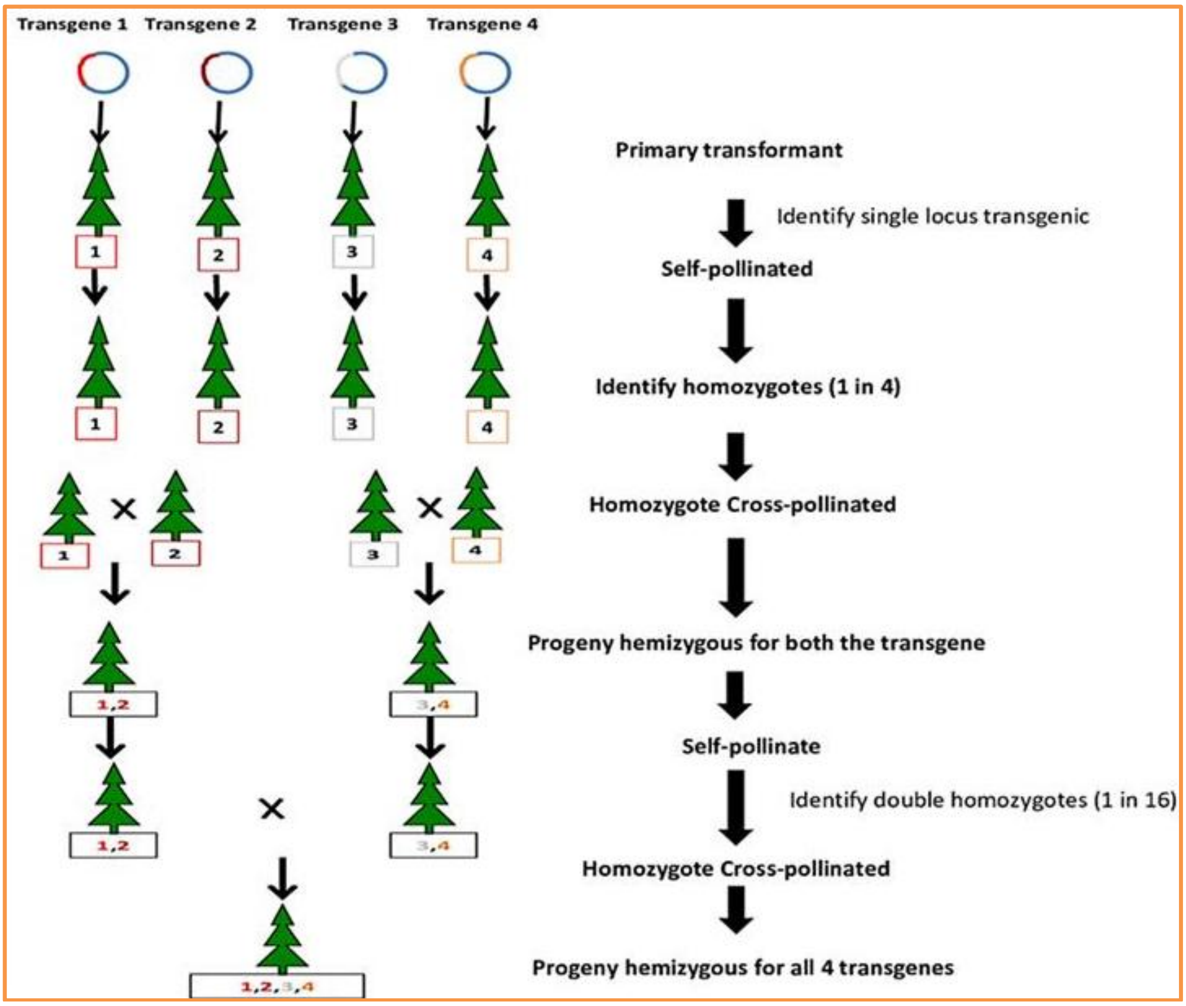
Gene Stacking V/s Gene Pyramiding

- **Gene Pyramiding** : assembling multiple desirable genes from multiple parents into a single genotype.
- **Gene Stacking** : combination of two or more transgenes of interest in the genome of the host plant.
- **Transgenic corn triple stacks**, for instance containing a corn root worm (CRW) protection trait (e.g., ***Cry3B(b)1***), a corn stalk boring insect control trait (e.g., ***Cry1A(b)***) and RR trait for herbicide tolerance.

Strategy for gene stacking



1. Sexual Hybridization : By crossing parents with different transgenes until the offspring have all the necessary genes, it is possible to create plants with several transgenes. One example is the cross breeding of tobacco to create a single plant with four genes encoding distinct immunoglobulin polypeptides, which results in the production of multiple IgA antibodies in plants (Ma *et al*. 1995). By crossing two genes for a bacterial organic mercury detoxification pathway organomecurial lyase, *merB*, and mercury reductase, *merA* in Arabidopsis, plants expressing both genes were able to grow on a methyl mercury concentration that was 50 times higher than that of wild type plants (Bizily *et al*., 2000).



Limitation:

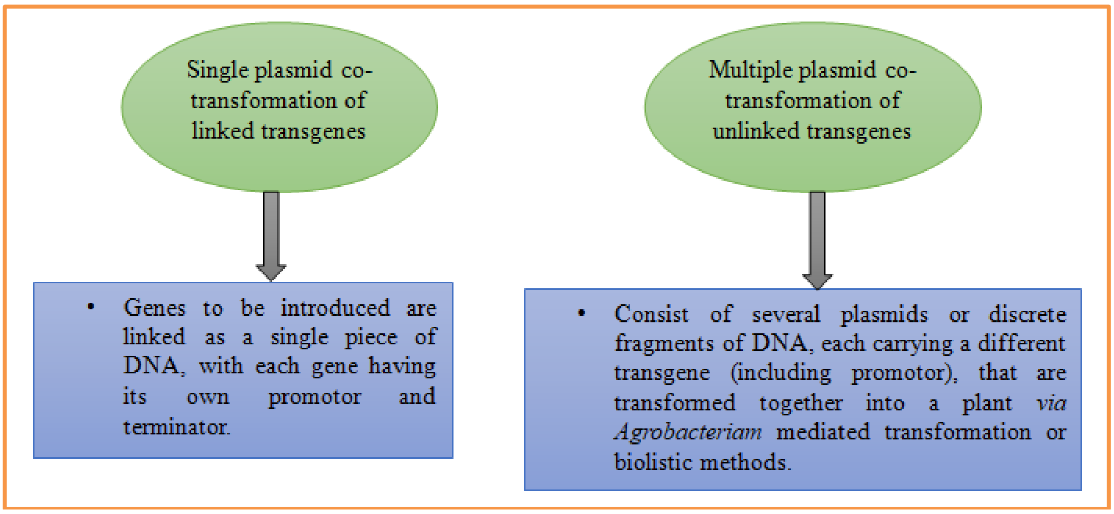
- During segregation, the introduced transgene will be positioned at various random locations throughout the plant genome.
- The size of the breeding population would double with each unlinked transgene added.
- Time-consuming and labour intensive.

2. Re-Transformation : A plant carrying a transgenic is transmitted again with another gene throughout this process. events with several traits or a combination of traits and distinct inserts. Iterative events using distinct insert transformation with vectors carrying various transgenes/traits resulted in GM plants. Several loci had the transgenic inserts incorporated. In a single strain of Agrobacterium, multiple transgenes are either independently retained inside distinct strains or within distinct T-DNA.

Limitation :

- Transgene silencing may result from re-transformation.
- A variety of selectable marker genes are required in order to use a distinct one for every successive transformation.

3. Co-Transformation : Three insecticidal genes, the Bt genes cry1Ac and cry2A as well as the snowdrop lectin gene gna have also been simultaneously introduced into indica rice by co-transformation via particle bombardment (Maqbool *et al*. 2001). Transgenic plants with all three genes demonstrated notable levels of defense against the brown plant hopper, yellow stem borer, and rice leaf folder, three of the most serious insect pests of rice. .



Advantage :

- A single step that introduces the multiple effect gene.
- Multiple transgene integration results in fewer transformation events and less time consumption; transgenes typically co-integrate at the same chromosomal location.

Disadvantage :

- High copy number
- Transgene silence caused by many tandem repeats or inverted repeats
- Undesirable inclusion of complex T-DNA molecules from multiple sources

How selection is done?

- Iterative method: at the phenotypic level
- When for the different traits on the basis of performance and response towards the desired character.
- When for the same trait (e.g., disease) molecular marker level.
- Re-transformation/Co-transformation: selection mainly with the help of markers assisted selection
- Selection evaluation on the basis of phenotypic characters.

Importance of Gene Stacking in Crop Improvement

1. Enhanced Resistance to Biotic Stresses : Stacking genes for resistance to multiple pests protects crops from diverse insect species, reducing yield losses.

Combines genes for resistance to different pathogens, offering broader and more durable protection against diseases.

2. Improved Tolerance to Abiotic Stresses

- Drought Tolerance : Essential for improving crop performance in water-scarce regions.
- Heat and Cold Tolerance : Helps crops adapt to temperature extremes caused by climate change.
- Salt Tolerance : Enables cultivation in saline soils, expanding arable land availability.

3. Increased Crop Yield and Productivity : Gene stacking allows the inclusion of traits that enhance yield potential, such as improved photosynthesis or grain filling.

Resistance to pests, diseases and environmental stresses minimizes yield losses, ensuring more stable production.

4. Multiple Herbicide Tolerance : Stacked herbicide tolerance traits allow farmers to use multiple herbicides effectively, preventing the emergence of herbicide-resistant weeds. Facilitates easier and more efficient weed control with fewer resources.

5. Improved Nutritional and Quality Traits : Stacked genes can enhance the nutritional profile of crops, such as higher protein, vitamins or mineral content. Includes traits like better taste, texture and shelf life, catering to market and consumer preferences.

6. Cost Effective : Reduces the need for developing multiple single trait varieties, saving resources and time for breeders and farmers.

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