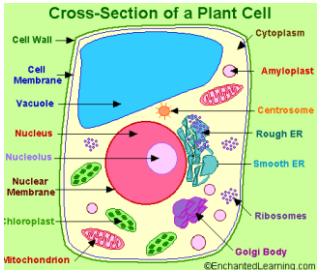
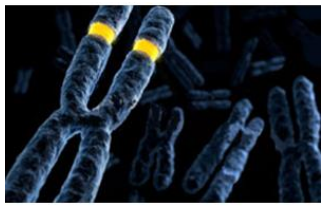
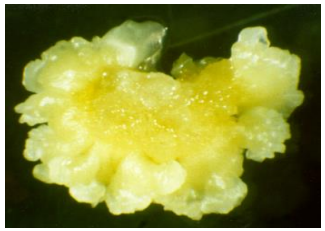
Selection and breeding process of the crops.
Breeding of stacked GM products and unintended effects

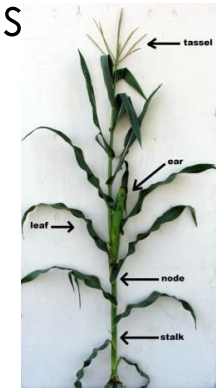
Critical steps in plant transformation







- Getting the gene into the plant genome
- Getting the plant cell to turn into a plant…
  - …that expresses the gene
- Getting a transformed plant to be fertile
- Getting the progeny to express the phenotype…
  - …without non-desired characteristics

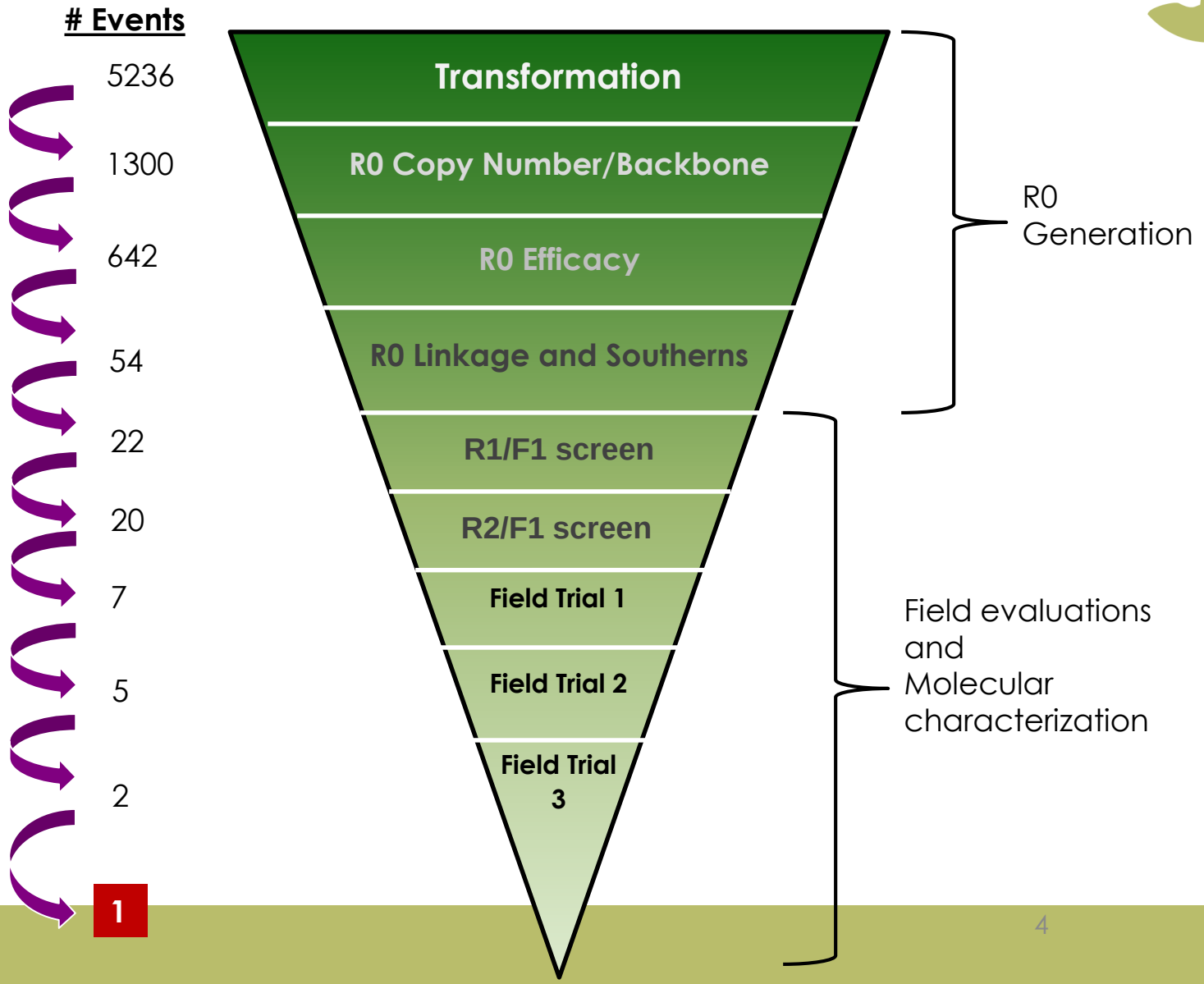


Criteria for selecting elite event

Criteria for event selection emphasize several factors:

- Insertion structure and structural fidelity of the DNA introduced through transformation relative to the transformation vector;
- Phenotypic expression of the trait according to desired threshold levels, tissue specificity, and timing in the plant life cycle;
- Consistent and reliable expression of the trait;
- Stable inheritance through numerous generations;
- Absence of negative or detrimental phenotypic effects.

Example of event selection process



Breeding stacks

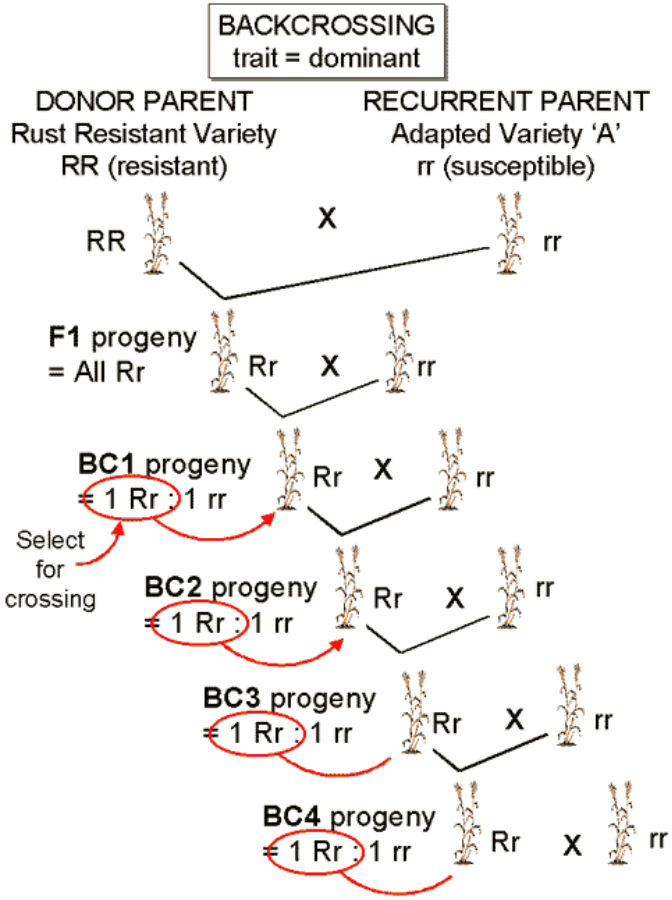
The overall objective of the breeding of stacked GM products is to integrate the specific transgenic events conferring the value-added trait phenotypes into the elite germplasm regaining the agronomic performance attributes of the target variety along with reliable expression of the value-added traits.

Breeding stacks process

The breeding of stacks process is comprised of four essential steps:

1. single event introgression,
2. event stacking
3. trait fixation
4. performance testing of various hybrids "versions" of a given target breeding stack

Single Event Integration by backcrossing

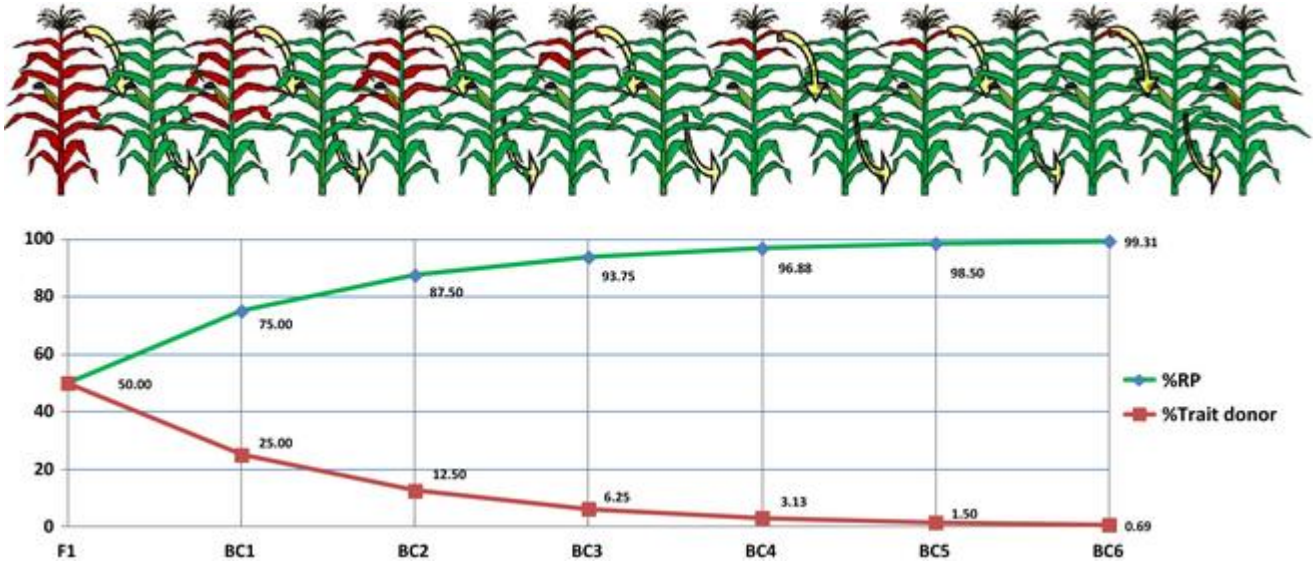


- **Backcross breeding is an effective method to transfer one or a few genes controlling a specific trait from one line into a second—usually elite—breeding line.**
- **The parent with the desired trait, called the donor parent, provides the desired trait but may not perform as well as an elite variety in other areas. The elite line, called the recurrent parent, usually performs well in all other areas.**

Example of a single introgression breeding process

generation	activity	seed outcome
1	cross event donor to RP	F1
2	select for event; BC to RP	BC1
3	select for event; BC to RP	BC2
4	select for event; BC to RP	BC3
5	select for event; BC to RP	BC4
6	select for event; BC to RP	BC5
7	select for event; BC to RP	BC6
8	self-pollinate	BC6S1
9	self-pollinate	BC6S2
10	identify line phenotypically similar to RP that stably expresses introduced trait	BC6S3
11	testcross to produce seed to evaluate agronomic performance of converted hybrid	BC6S3 testcross

Recovery of Recurrent Parent genotype by backcrossing



Single event introgression is conducted in parallel streams to convert the recurrent parent (RP) for individual events, with the primary objective of minimizing residual non-recurrent parent (NRP) germplasm, especially in the chromosomal proximity to the event (i.e. linkage drag).

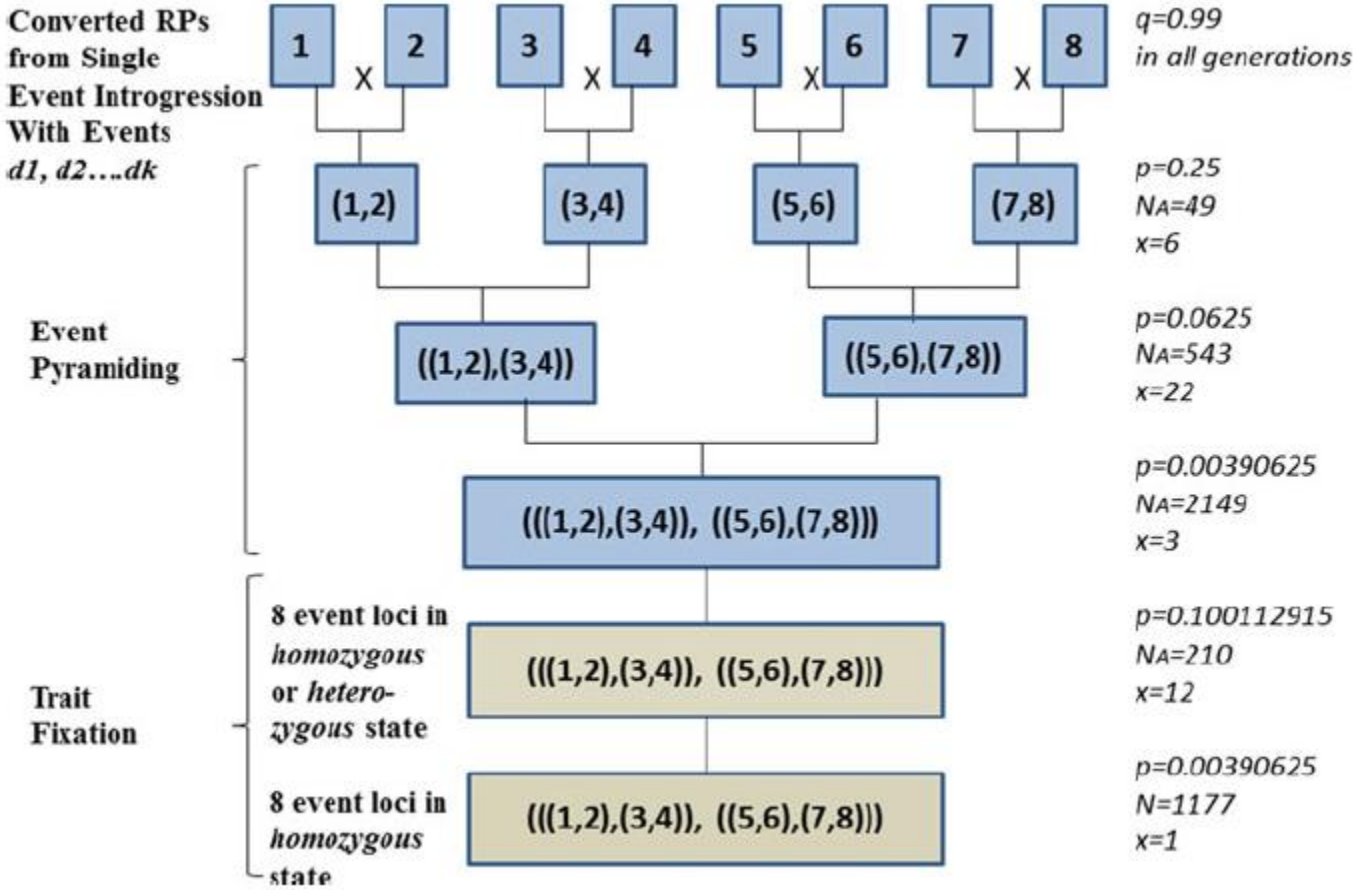
Event stacking and trait fixation

Technique of Self-Pollination. The tassel is covered with a bag to collect uncontaminated pollen

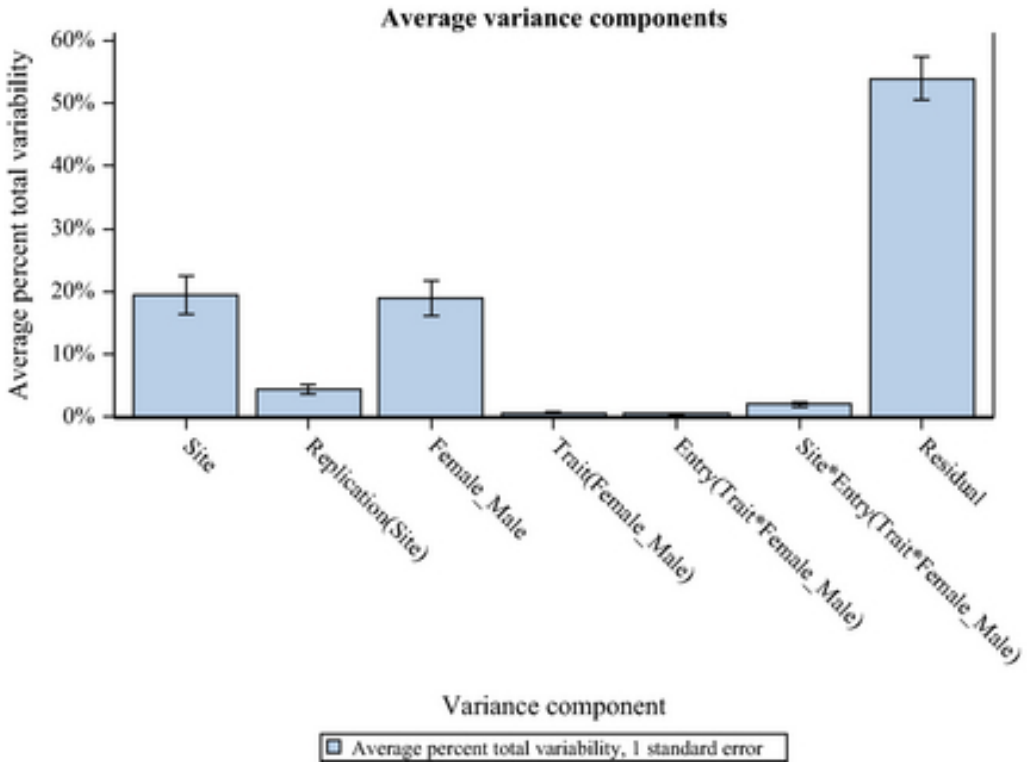
Technique of Self-Pollination. The ear shoot is covered before any silks appear

- The breeding goal for **event stacking** is to assemble all the specified events in the target variety by crossing single event conversions to create stacked versions of each variety with all events in a heterozygous state.
- **For trait fixation**, the breeding goal is to recover at least one line which is homozygous for all event loci to ensure stable expression of value-added traits.
- In order to minimize the risk of failure in recovering the target hybrid performance, typically multiple versions of conversions are generated and yield-tested

Overview of the event stacking and Trait fixation



Effects of Backcrossing vs transformation



The largest sources of variation are the genetic background of the different conventional inbred lines (males and females) used to generate the maize hybrids and location.

Differences between near-isogenic GM and conventional maize hybrids are associated with backcrossing practices in conventional breeding

Compositional differences between near-isogenic GM and conventional maize hybrids are associated with backcrossing practices in conventional breed

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History of Safe Use of Breeding

- Historically the focus of development for most crops has been on the introduction of desirable characteristics for producers, processors and end-users, without compromising pre-existing characteristics, and while maintaining uniformity.
- Examples of adverse effects are extremely rare, and when they have occurred, they have all involved known compounds, not novel ones (Steiner et al. 2013).

Plant breeding and selection practices minimizes advancement of unintended effects

Step 1
Selection

GM Event Selection

n000

1

Unintended DNA changes tested and eliminated

Step 2
Trait integration

Breeding GM Plant Into Elite Germplasm

>90% of DNA from transformed plant is removed

Step 3
Commercial breeding

Developing hybrids to grow in many geographies

An additional elimination of DNA from the transformed plant in final seed product

Conclusions

The end result of the selection process is that unintended traits which may have occurred are removed.

Potential differences between genetically modified (GM) and non-GM comparators cannot be attributed unequivocally to the GM trait.

Safety assessment of breeding stacks can be more effectively addressed through targeted hypothesis-driven evaluations than by large-scale prescriptive comparative studies.

Literature

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