Recent developments – New plant breeding techniques and uses of plants

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Innovation in plant breeding : Special features

- Seed, a vehicle of innovation
  - Self-replication of living organisms
  - Gene flow and recombination driving constant variation

- Challenges for plant breeding :
  - *Creating* novel combinations of alleles
  - *Fixing* desirable combinations for market release
  - *Controlling* gene flow (specially for novel, non food traits)

Drives for changes

Genetic resources

↓ ← Gene discovery (the ‘omics)

New breeding techniques

Crop varieties

↓ ← New propagation techniques

Seeds

↓

Plant products ← New demands from industry and consumer

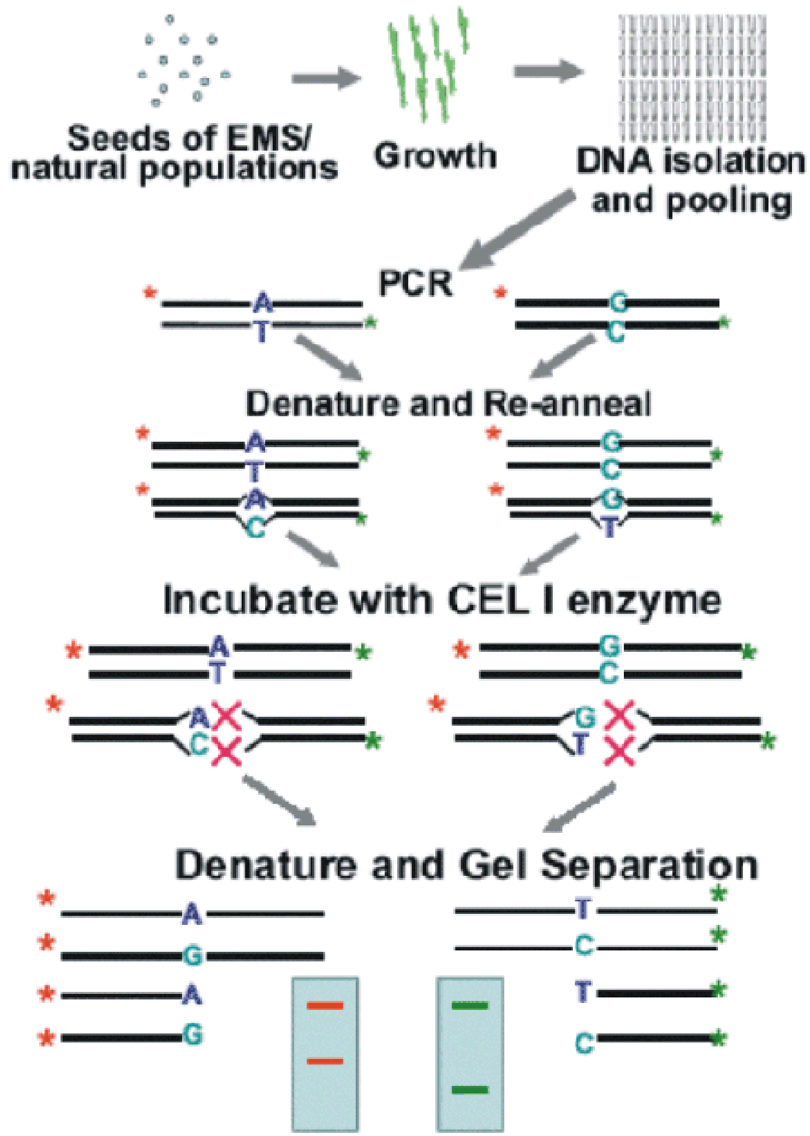
↓

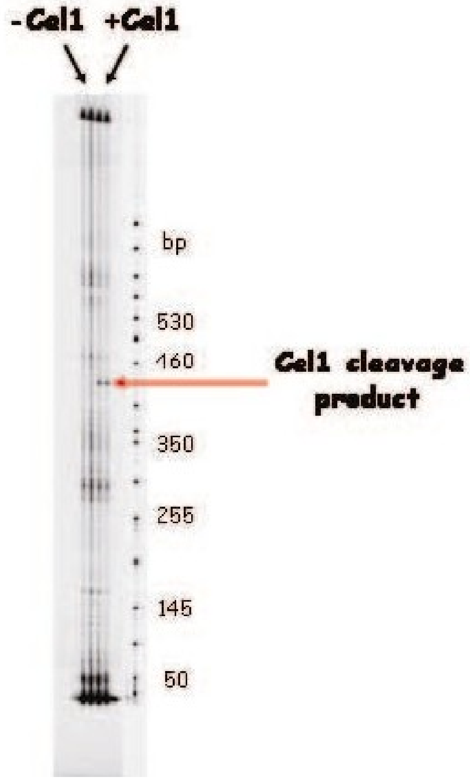
The field environment ← New demands from the society

Exploiting and enhancing genetic variation : overview

Objective	Activity	New technologies
Identifying existing genetic variation	Genetic maps, Molecular markers	High throughput technologies (from genomics to phenomics)
Enhancing genetic variation	Random and targeted mutagenesis	TILLING (for *Targeted induced local lesions in the genome),* Oligonucleotide-mediated site-specific mutagenesis
Extending gene recombination	Gene isolation and transfer	Recombinant DNA, GM technology

TILLING (*Targeted induced local lesions in the genome*) : Combining random mutagenesis with the highly efficient molecular identification of mutants in targeted genes





The next step : creating new alleles by site-specific mutations in targeted genes

Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 8774–8778, July 1999
Plant Biology

A tool for functional plant genomics: Chimeric RNA/DNA oligonucleotides cause *in vivo* gene-specific mutations

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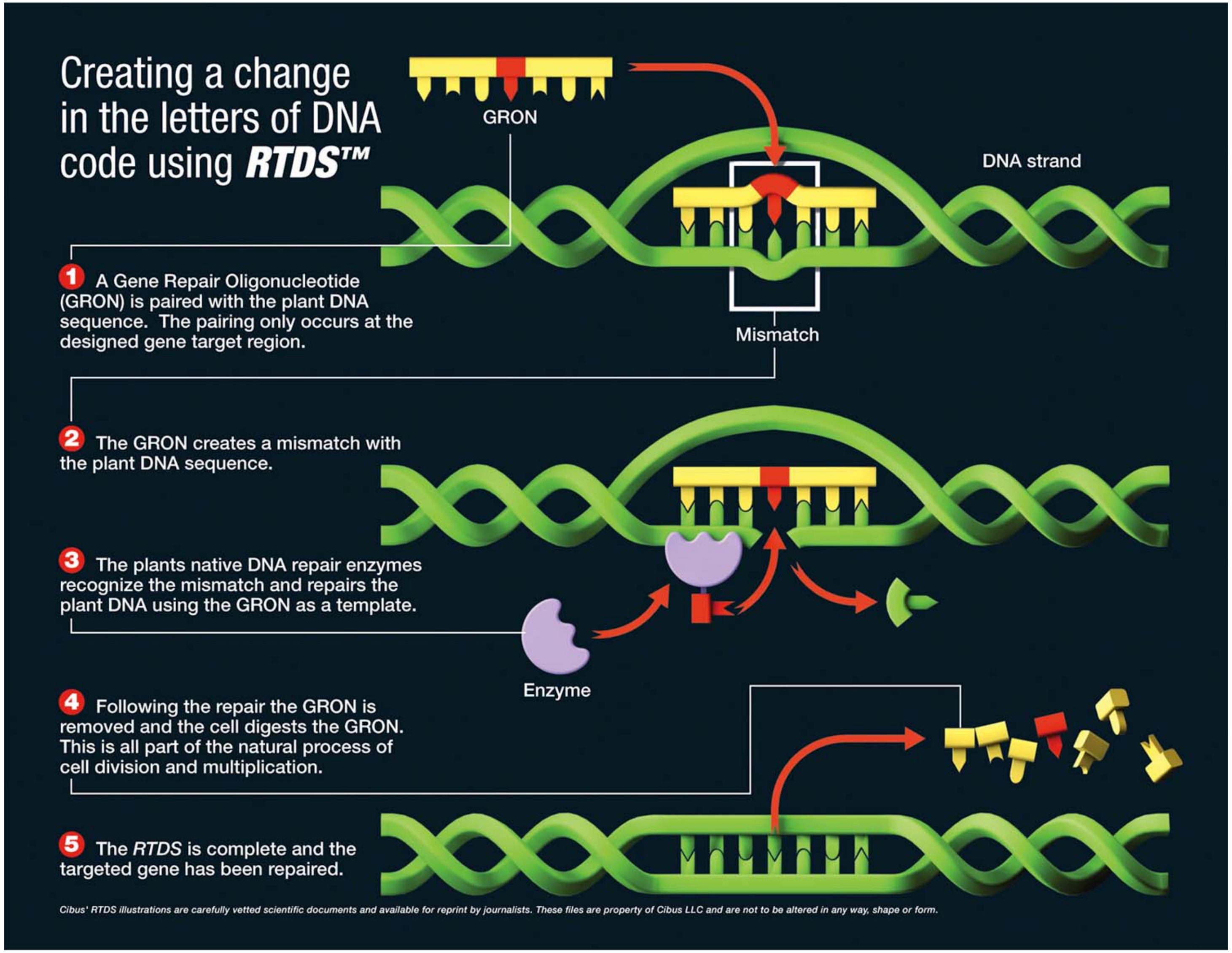
Achievements on agricultural traits/crop plants :

Chlorsolfuron resistance in Rice and Tobacco, imidazolinone resistance in Maize

How it works : RNA/DNA oligonucleotides are delivered to plant cells, invading genomic DNA and triggering the endogenous DNA repair machinery to produce site-specific DNA substitutions/ deletions.



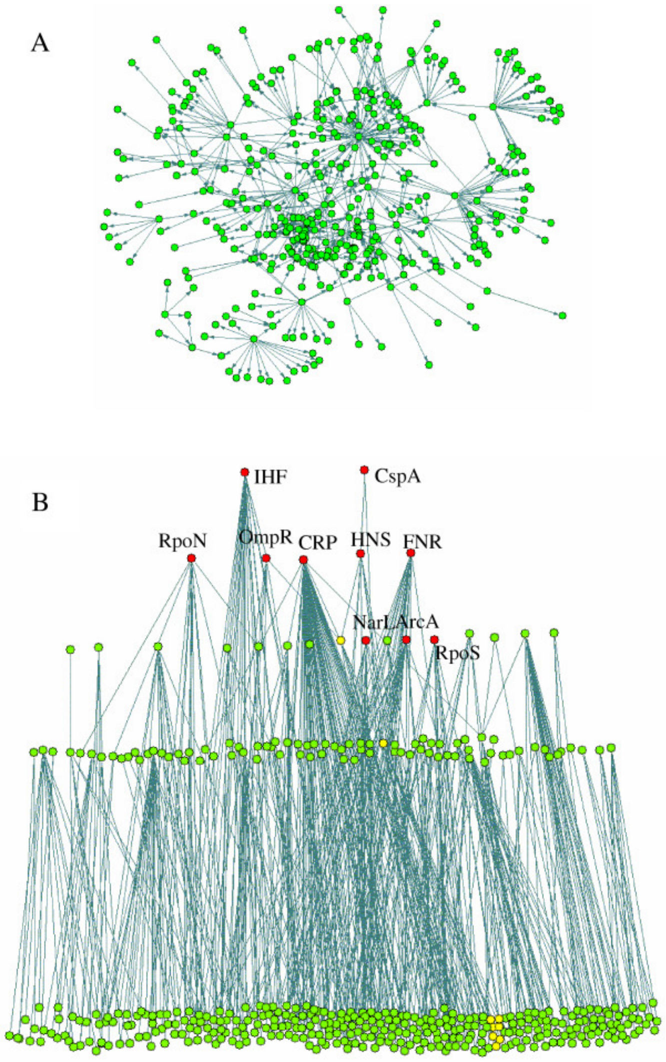
Which future for oligonucleotide-mediated, site-specific mutagenesis ?

A promising tool for the creation of new alleles in targeted genes, but …

1. low frequency mutation, asking for efficient selection (positive selection using dominant mutated alleles),
2. single cell mutation to be conducted to whole plant regeneration (pratictal limitations and risks of somaclonal variation),
3. biolistic delivery of the mutagenic oligonucleotides to plant cells, with possible unintended (and unknown) effects.

Fixing traits = Fixing gene combinations ? Conceptual challenges from the 'omics' era

- *Epistasis* : the gene is part of an interaction network (the 'interactome'), with complex and moving hierarchies.
- *Epigenetics* : gene regulation, not only the gene itself, is heritable.
- *Regulatory RNAs* : extensive transcription of 'non-coding' DNA plays an essential role in gene regulation.



A new paradigm : regulatory RNAs transcribed from intergenic, non-coding DNA control gene expression ('gene silencing').

Noncoding Transcription by RNA Polymerase Pol IVb/Pol V Mediates Transcriptional Silencing of Overlapping and Adjacent Genes

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(Cell (2008), 135: 635-648)

Conclusions :

1. Agronomic traits are controlled by genes, but also by gene networks and by heritable and non heritable gene regulators.
2. Empirical selection (based on phenotype) is supported by genetic and epigenetic determinants of emerging complexity.
3. Gene regulations, not only gene combinations, must be captured by plant breeding and by the selection of plant varieties.

Enhancing gene recombination : GM technology

GMO regulation in the EU is based on the process of gene recombination, not on the final characteristics of the organism.

→ A debate : Can *cis*-genics (the integration of ‘already-there’ DNA) bypass the regulation on *trans*-genics (the integration of ‘exotic’ DNA) ?

GM technology in Plants : Some current technical limitations

1. DNA-addition only (no DNA replacement)

   → Still far from ‘gene targeting’ in crop plants …

2. Non-targeted integration in the genome, with possible unintended effects on plant phenotype

   → Agronomic testing and the EU risk assessment procedure address the point.

3. Non-targeted integration with unpredictable effects on transgene expression (‘position effects’)

   → Agronomic testing and the EU risk assessment procedure address the point.

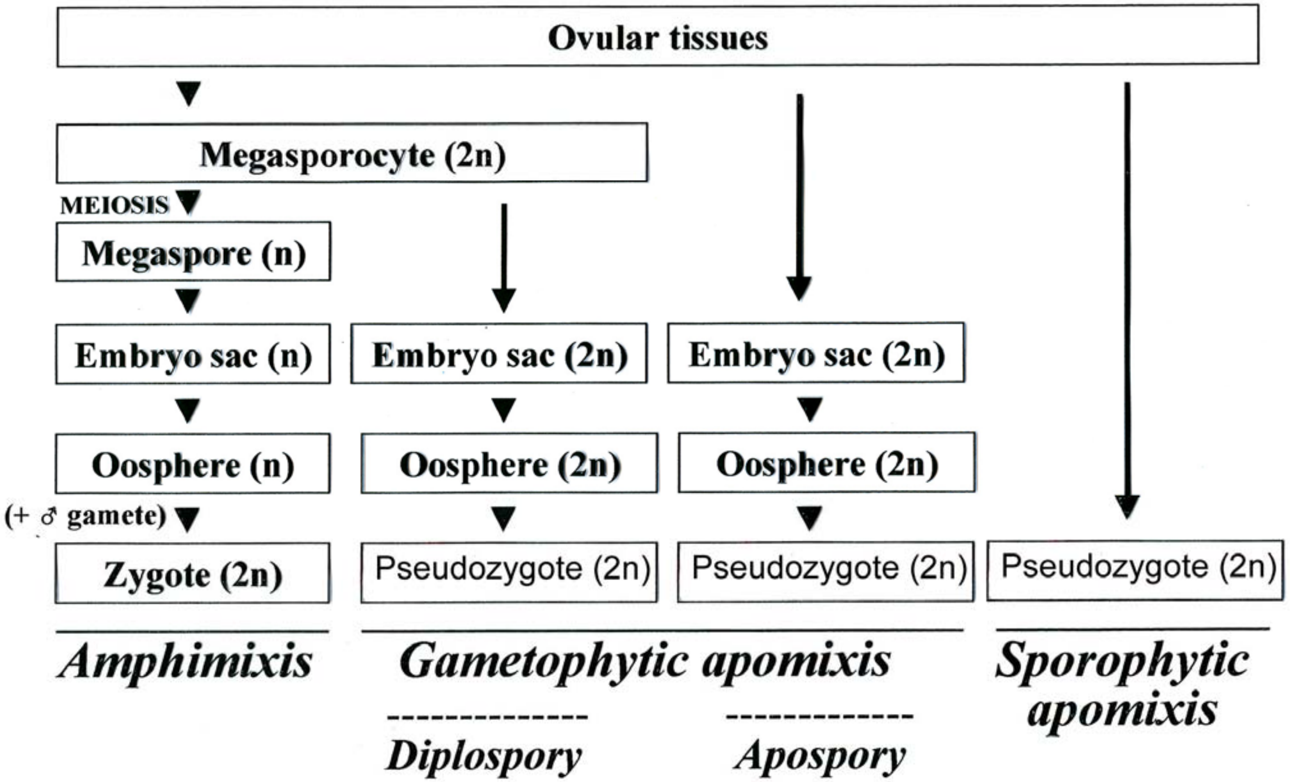
4. Low efficiency transformation may require selection markers (antibiotics resistance) raising safety concerns.

   → Alternatives to the use and persistence of Antibiotics resistance marker genes in the marketed GMPs exist.

   → Extensive risk assessment failed to identify any adverse effects until now.

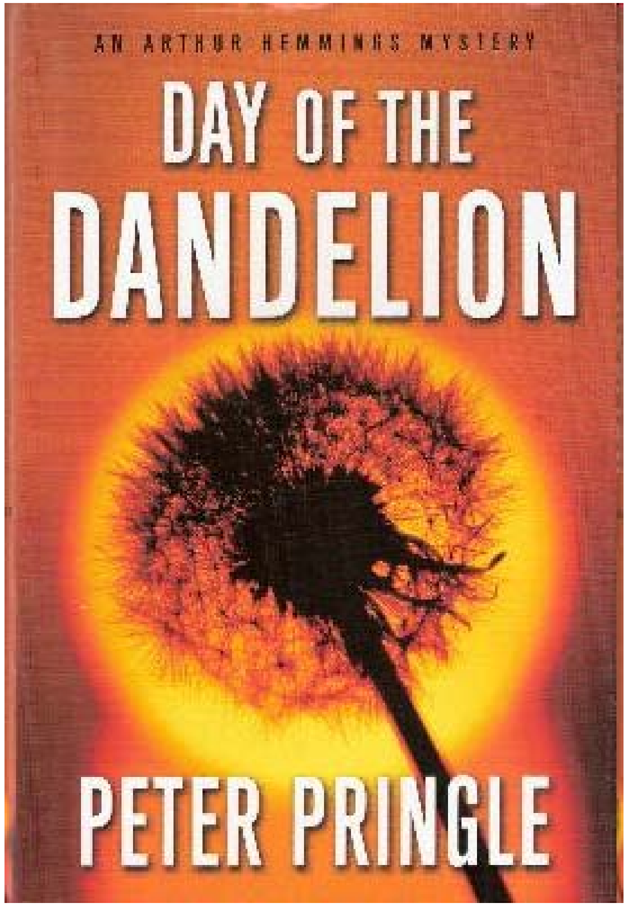
Fixing the trait in seed populations : A role for apomixis ?

Apomixis is the production of viable seeds without fertilization.

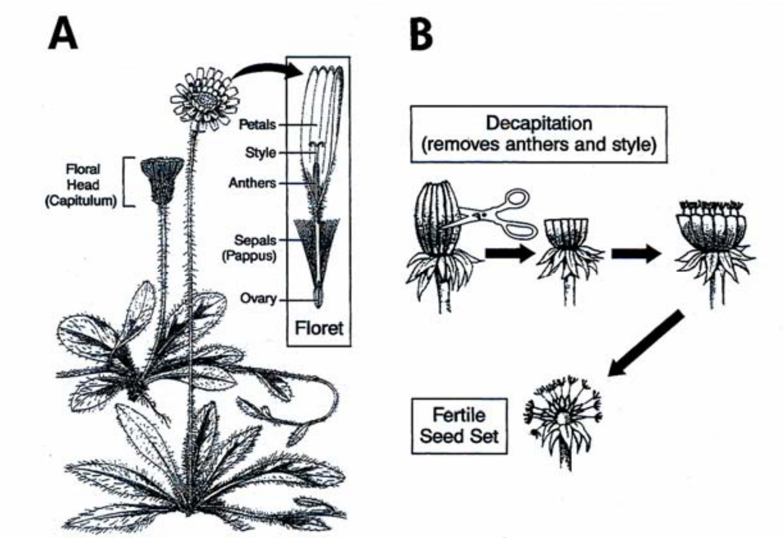


The quest of apomixis …

- *« Apomixis in agriculture : the quest of clonal seeds »*
  (Spillane et al (2001) 14: 179-187)
- *« Apomixis : The asexual Revolution »*
  (Vielle Calzada et al, Science (1996) 274, 1322-1323)
- Apomixis, the topic of a
  « botanical thriller » by Peter Pringle (2007) …



- Apomixis is found in several botanical families, yet it is missing in important crop families and genera.



Koltunow *et al.* Plant Physiol. (1995) 108: 1345-1352

- Genes are being discovered in model plants, which control the apomictic development of embryos and endosperm. This paves to way to the genetic engineering of apomixis into crop plants.

Gamete formation without meiosis in *Arabidopsis*

Maruthachalam Ravi[1], Mohan P. A. Marimuthu[1] & Imran Siddiqi[1]

Ravi *et al.* Nature (2008) 451: 1121-1124

Controlling gene flow

- Molecular pharming needs containment.
- Biological containment is an option.
- Biological containment may be achieved by :
  - Male sterility,
  - Seed sterility,
  - Cleistogamy,
  - Inducible promoters of transgene expression,
  - Transgenic mitigation,
  - Maternal inheritance,
  - Etc.
- *« By choosing the right plant, the development of gene-containment mechanisms may be unnecessary. »* (In : Johnson and Dallimore, 2002, Nature Biotechnology, 20: 871)

Genetic transformation of the plastid genome combines efficient expression of ectopic proteins with reduced gene flow via the pollen.

TABLE 2 | Crops in which successful chloroplast transformation has been reported.

Crop	Site of integration	Marker/gene of interest
Carrot	*trn*I/*trn*A	*aad*A, *badh*
Cauliflower	*acc*D/*rbc*L	*aad*A
Cotton	*trn*I/*trn*A	*aph*A6, *npt*II
Lettuce	*trn*I/*trn*A	*aad*A, *gfp*
Lettuce	*acc*D/*rbc*L	*aad*A, *gfp*
Lettuce	*trn*I/*trn*A	*aad*A, *CTB-Pris*
Oilseed rape	*rps*7/*ndh*B	*aad*A, *cry*1Aa10
Petunia	*acc*D/*rbc*L	*aad*A, *uid*A
Poplar	*acc*D/*rbc*L	*aad*A/*gfp*
Potato	*acc*D/*rbc*L *rrn*16 *trn*V/3′*rps*7/12	*aad*A, *gfp*
Potato	*acc*D/*rbc*L *rrn*16 *trn*V/3′*rps*7/12	*aad*A, *gfp*
Rice	*trn*I/*trn*A	*aad*A, *gfp*
Soybean	*trn*V/3′*rps*7/12	*aad*A
Tobacco	*acc*D/*rbc*L	*aad*A
Tobacco	*trn*I/*trn*A	*aad*A, *aro*A
Tomato	*trn*fM/*trn*G	*aad*A

↑

Verma *et al.* Nature Protocols (2008) 3: 739-758

Site-specific integration !

Conclusions :

- Chemically-induced Mutants in targeted genes can be selected with increasing efficiency (TILLING).
- Targeted genes can be mutated in a site-specific way (for some traits).
- Some gene regulators are heritable and new molecular insight is being gained on ‘epigenetics’.
- Engineering of apomixis in crop plants is on the way.
- GM technology is still developing, with plastid transformation as a promising tool for the eco-friendly production of bioactive proteins.