Code	Name	Type	Promoter, other	Terminator	Copies	Form
cry1Ab	Cry1Ab delta-endotoxin (*Btk* HD-1)	IR	enhanced CaMV 35S, maize HSP70 intron	None. Lost through 3' truncation during integration	1	Truncated

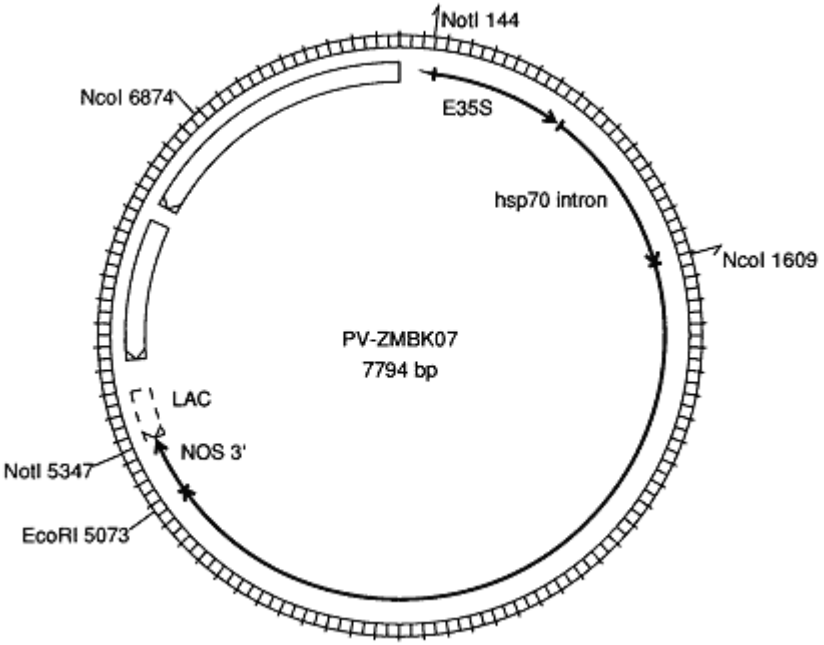
Maize line MON 810 was produced by biolistic transformation of maize genotype Hi-II with a mixture of plasmid DNAs, PV-ZMBK07 and PV-ZMGT10. The PV-ZMBK07 plasmid contained the *cry1Ab* gene and PV-ZMGT10 plasmid contained the CP4 EPSPS and *gox* genes. Both plasmids contained the *nptII* gene under the control of a bacterial promoter required for selection of bacteria containing either plasmid, and an origin of replication from a pUC plasmid (ori-pUC) required for replication of the plasmids in bacteria.

http://cera.projectstagingserver.com/GmCropDatabaseEvent/event/9

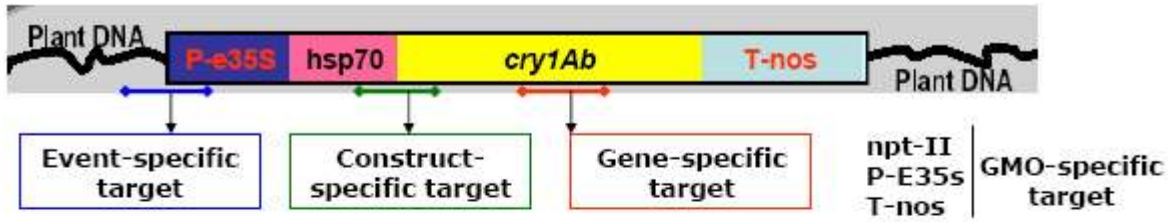
CaMV Enhanced 35S promoter
0.61 Kb

Hsp70 intron
0.80 Kb

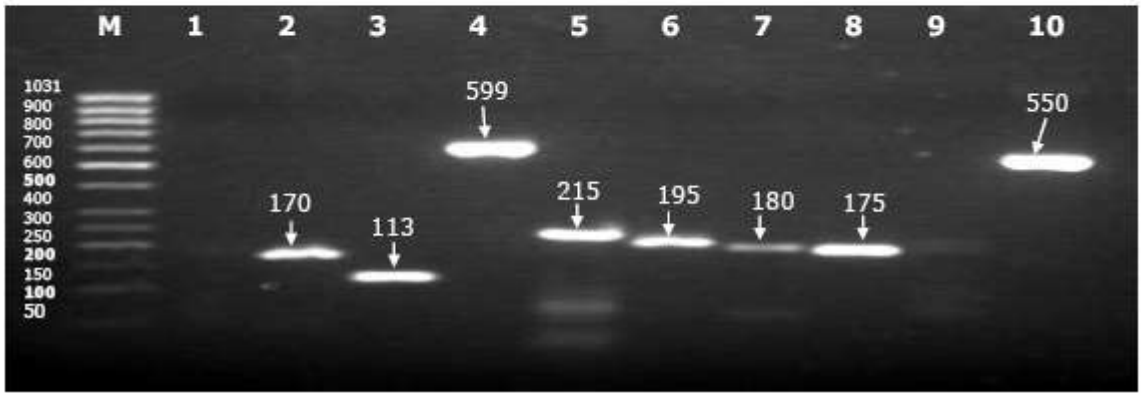
Cry1Ab
3.46 Kb



A



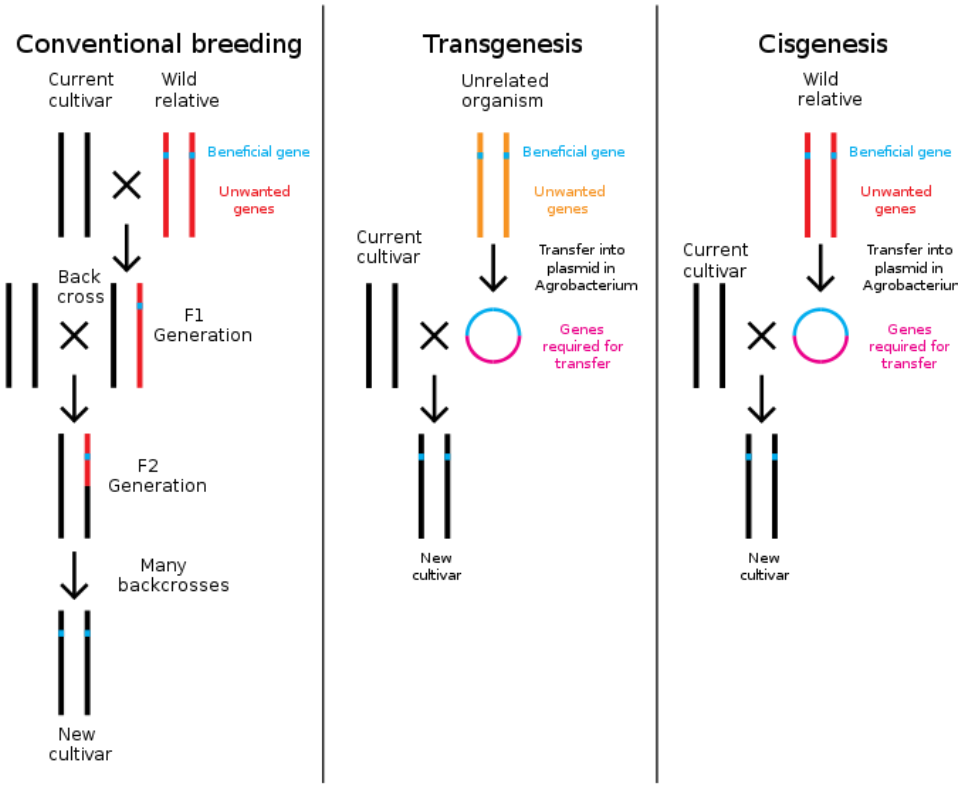
B

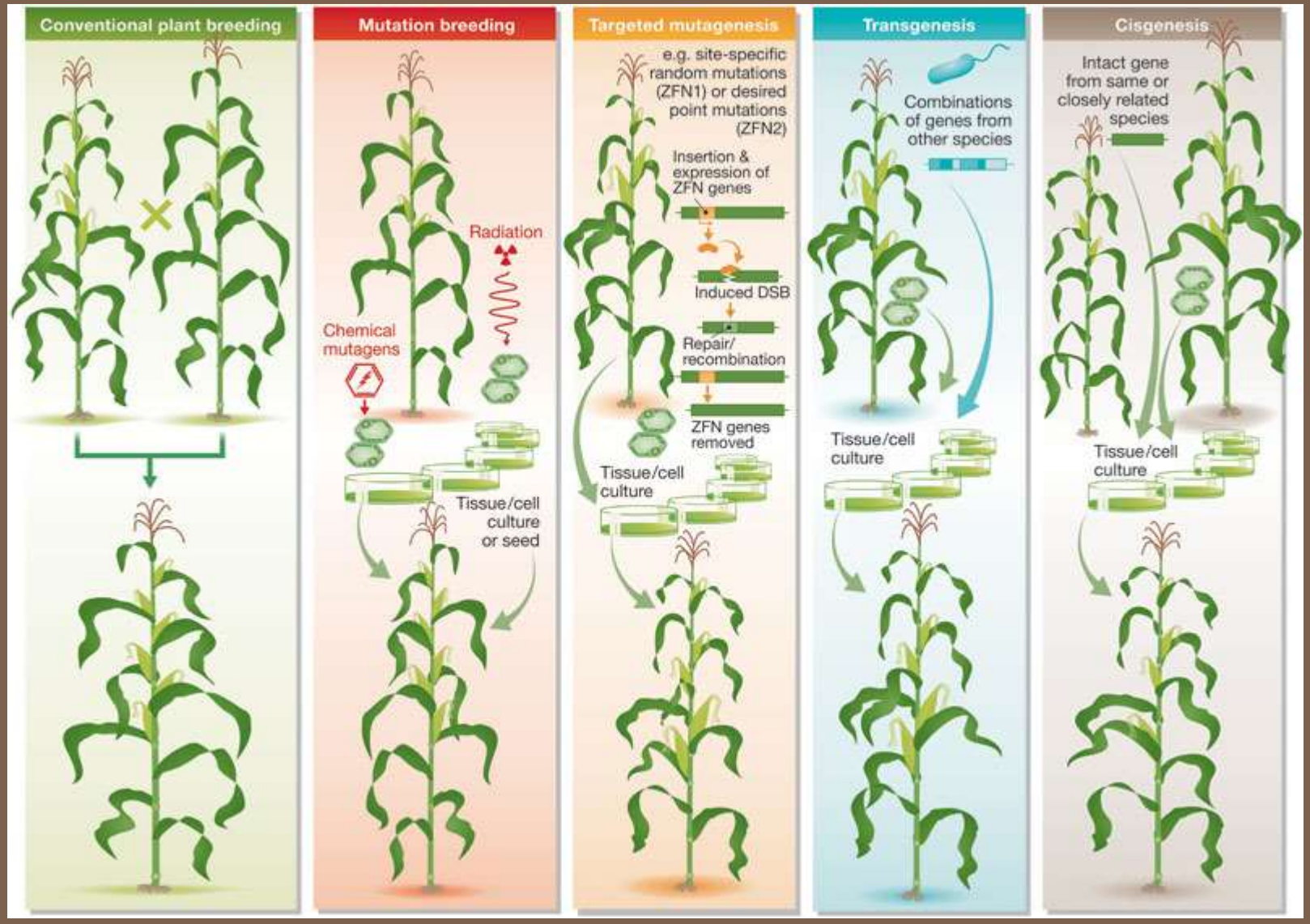


Protocol Exchange (2007) doi:10.1038/nprot.2007.440

Trendi v rastlinski biotehnologiji

- Transgeneza
- Intrageneza
- Cisgeneza
- Cepljenje z GSR
- Agroinfiltracija
- Od RNA odvisna metilacija DNA




Conventional plant breeding
Mutation breeding
Radiation
Chemical mutagens
Tissue/cell culture or seed
Targeted mutagenesis
e.g. site-specific random mutations (ZFN1) or desired point mutations (ZFN2)
Insertion & expression of ZFN genes
Induced DSB
Repair/ recombination
ZFN genes removed
Tissue/cell culture
Transgenesis
Combinations of genes from other species
Tissue/cell culture
Cisgenesis
Intact gene from same or closely related species
Tissue/cell culture

Cisgeneza je genetsko spreminjanje rastlin izključno s cisgeni.

Cisgen je naravni gen, ki zapisuje za neko lastnost in izhaja iz iste rastline ali iz genetsko kompatibilne donorske rastline, ki jo je dovoljeno uporabljati tudi v konvencionalnem žlahtnenju. Ti geni sodijo v genski sklad konvencionalnih žlahtniteljev.

Cisgenska rastlina ne vsebuje tujih genov.

Intrageneza je genetsko spreminjanje prejemnega organizma, ki privede do kombinacije različnih genskih fragmentov donorskega organizma (enega ali več), ki je ista rastlina ali je genetsko kompatibilna s prejemno. Genski fragmenti so lahko razporejeni smerno ali protismerno glede na orientacijo v donorskem organizmu.

Transgen je gen iz vrste, ki se ne more križati z vrsto, ki jo spreminjamo, ali pa gre za sintenski gen. Tak gen prištevamo v nov genski sklad za žlahtnenje rastlin.

Transgenska rastlina je GS rastlina, ki vsebuje transgene.

Tradicionalno ali konvencionalno žlantnenje uporablja klasične in sodobne pristope in tehnike vključno z biotehnološkimi, a ne vključuje genskih sprememb. Obstaja vrsta tehnik, ki jih nimamo za gensko spreminjanje oz. so izvzete iz zakonodaje o GSO.

http://www.cisgenesis.com/content/view/2/25/lang,english/

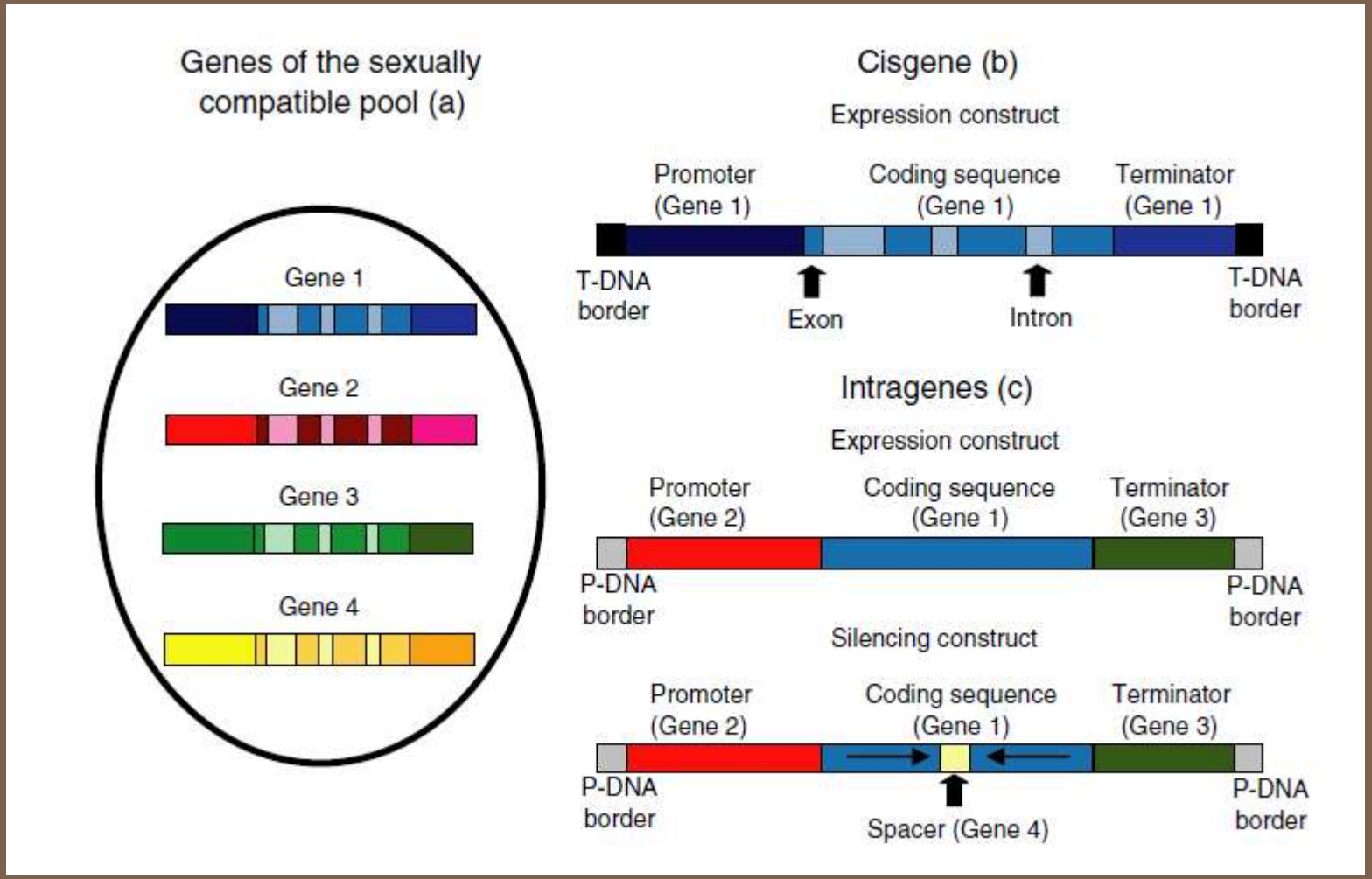


Figure 1 Illustration of cisgene and intragene constructs as defined by Schouten *et al.*, (2006a) and Rommens (2004), respectively. The cisgene is an identical copy of a gene from the sexually compatible pool including promoter, introns and terminator (a,b). When using *Agrobacterium*-mediated transformation the cisgene is inserted within *Agrobacterium*-derived T-DNA borders. Intragenesis allows *in vitro* recombination of elements isolated from different genes within the sexually compatible gene pool (a,c). Furthermore, there is no requirement for introns and cDNA or fragments of genes can be used. Thus, both expression and silencing intragenic constructs can be designed. According to the definition of Rommens (2004), the intragene should be inserted within borders isolated from the sexually compatible DNA pool (P-DNA borders), when using *Agrobacterium*-mediated transformation.

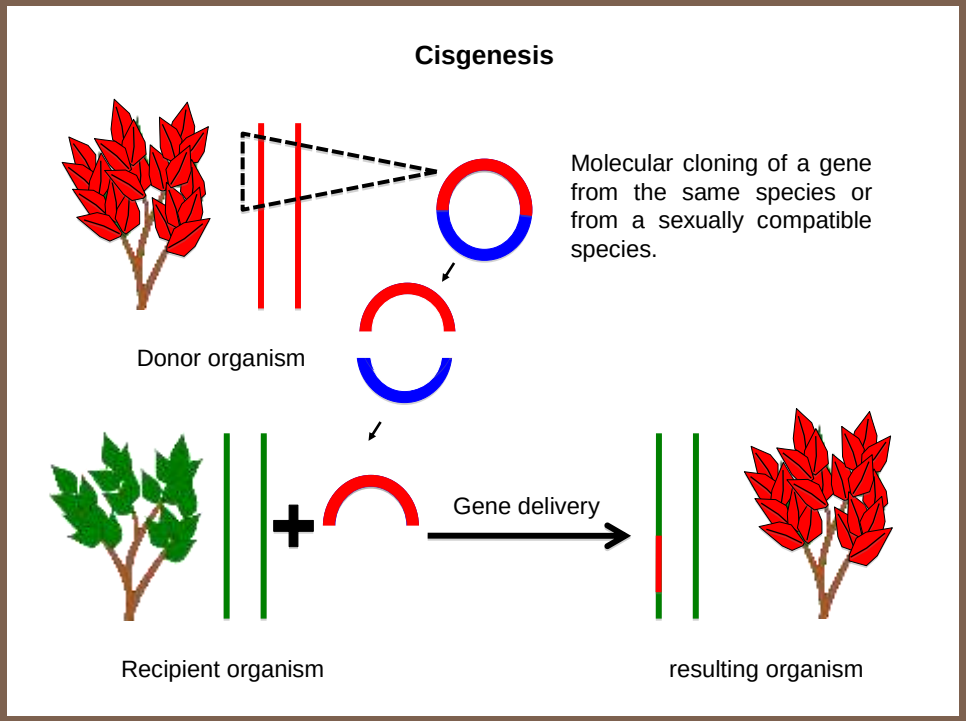

Cisgenesis
Molecular cloning of a gene from the same species or from a sexually compatible species.
Donor organism
Gene delivery
Recipient organism
resulting organism

Table 2 Intragenic/cisgenic crops developed or currently under development

	Type	Prom./term./spacer from	Gene	Trait	Authors
Intragenesis					
Potato	Silencing	*GBSS/nos* Spacer: *GBSS*-fragment *GBSS/GBSS* Spacer: *GBSS*-fragment	*GBSS*	High amylopectin	de Vetten *et al.*, (2003)
Potato	Silencing	*GBSS/Ubi3* Spacer: *Ubi7*-fragment	*Ppo*	Preventing black spot bruise	Rommens *et al.*, (2004)
Potato	Silencing	*GBSS/Ubi3* Spacer: *Ubi7*-fragment	*Ppo, R1, PhL*	Preventing black spot bruise. Limiting cold-induced degradation of starch. Limit acrylamide in French Fries	Rommens *et al.*, (2006)
Potato	Silencing	Prom.*Agp*/Prom.*GBSS** Spacer: *Ubi7*-fragment	*StAs1, StAS2*	Limit acrylamide in French Fries	Rommens *et al.*, (2008)
Potato	Silencing	Prom.*Agp*/Prom.*GBSS** Spacer: *Ubi7*-fragment	*StAs1*	Limit acrylamide in French Fries	Chawla *et al.*, (2012)
Apple	Expression	*RbcS/RbcS*	*HcrVf2*	Scab resistance	Joshi *et al.*, (2011)
Strawberry	Overexpression	*FaExp2/FaExp2*	*PGIP*	Gray mould resistance	Schaart, (2004)
Alfalfa	Silencing	Prom*PetE*/Prom*PetE** Spacer: *Comt*-fragment	*Comt*	Reduced levels of lignin	Weeks *et al.*, (2008)
Perennial ryegrass	Overexpression	n.m. but from species itself	*Lpvp1*	Drought tolerance	Bajaj *et al.*, (2008)
Cisgenesis					
Potato	Expression	Gene's own	*R-genes*	Late blight resistance	Haverkort *et al.*, (2009)
Apple	Expression	Gene's own	*HcrVf2*	Scab resistance	Vanblaere *et al.*, (2011)
Grapevine	Expression	35S-CMV/35S-CMV	*VVTL-1, NtpII*	Fungal disease resistance	Dhekney *et al.*, (2011)
Poplar	Overexpression	Gene's own	Genes involved in growth, *PAT*	Different growth types	Han *et al.*, (2011)
Barley	Overexpression	Gene's own	*HvPAPhy_a*	Improved grain phytase activity	Holme *et al.*, (2012)
Durum wheat	Expression	Gene's own	*1Dy10*	Improved baking quality	Gadaleta *et al.*, (2008)

*This type of 'convergent transcription' silencing construct with two promoters was shown to be very efficient (Yan *et al.*, 2006). n.m.: not mentioned.

White Paper

Cisgenic crops should not be under the European GMO Regulation

Definitions

Conventional plant breeding uses only genes from the crop species itself or from crossable plants. Cisgenesis uses these same genes. Cisgenesis is the genetic modification of a recipient organism with a gene from a crossable organism (same species or closely related species). This gene has exactly the same DNA sequence as in the crossable donor organism, and includes its native promoter in the natural orientation. Cisgenic plants can harbour one or more cisgenes, but they do not contain any parts of transgenes.[1]

Socio-economical reasons for cisgenesis

There are several advantages of cisgenesis compared to conventional breeding:

1. The original genetic make-up of the plant variety is maintained. In case of crossing, the genome of an offspring plant is a mixture of the genomes of the parental plants. The genetic-make up of the progeny plant differs from its parents and is in fact a mixture of the parents' genomes. However, sometimes the genetic make-up of current varieties must be preserved. E.g., when a well-known grape variety (such as Merlot or Cabernet sauvignon) is crossed with a disease resistant grape plant, the genetic make-up of the progeny plants will never be the same as of the well-known parental variety. Therefore cross breeding cannot make well-known cultivars resistant to diseases and pests. This is especially a problem for self-incompatible plants that are vegetatively propagated, such as grape, potato, apple, banana, strawberry, etc. However, cisgenesis can add disease resistance genes to a well-known variety without disturbing the genetic make-up of that variety. Therefore, cisgenesis can turn well-known susceptible varieties into resistant ones, yet keeping their specific quality traits.

2. Strong reduction of pesticide input. Cisgenesis is mainly applied to add disease resistance genes to susceptible varieties [2]. The aim is a strong reduction of pesticide input. This reduces costs for growers, reduces pesticide residuals on plant products, which is preferred by consumers, lowers the environmental footprint, and supports sustainable agriculture.However, if the GMO Regulation would cover cisgenic crops, then this innovation would be halted, in spite of the clear advantages of cisgenesis.

3. Gain of time. During crosses in conventional plant breeding, hundreds of undesired alleles are inherited to the progeny, leading to lower quality. This is called genetic drag. In order to remove these undesired alleles, several breeding generations are required. Cisgenesis prevents genetic drag. Only the desired gene or allele is transferred. This saves time. In case of e.g. apple-breeding introgression of a disease resistance gene

GMO Potato: Cisgenesis is still Genetic Modification with All the Attendant Risks

Posted August 16, 2013 by I-SIS & filed under Biodiversity, GMOs, Health & Disease, Soil Erosion & Contamination, Water Contamination & Loss

The new trial on GM blight-resistant potato is being sold as cisgenic technology to confuse the public, but it is still genetic modification with all the attendant risks — most of which are not addressed.

by Dr Eva Sirinathsinghji

A new genetically modified (GM) 'blight-resistant' potato is currently being tested in a three year large-scale field trial in Carlow, Ireland conducted by the Irish government in collaboration with the Dutch Wangeningen University [1]. Potato blight, a devastating fungal disease caused by Phytophthora infestans, was responsible for the terrible Irish potato famine in 1845-52. The trial, started this summer, is a follow up study of the initial small-scale field trial performed in 2012.

With scepticism and distrust towards GM crops prevalent in Ireland, a country known for environmental consciousness, proponents of the new trial are attempting to further blur the scientific facts associated with the blight-resistant potatoes. These potatoes are being dubbed 'cisgenic' instead of 'transgenic', claiming that cisgenesis is the process of transferring a gene from one species to another sexually compatible one. Wageningen University and collaborating organisations have even gone to the lengths of publishing a website with spurious definitions in order to spread the confusion as far as possible (see below) [2].

So what exactly does cisgenesis and transgenesis mean? Transgenesis can be defined as the transfer of foreign genetic material into an organism by genetic engineering techniques. As is the case with this GM crop, the host potato species (Solanum tuberosum cv. Desiree) is different from the wild relative species Solanum venturii that provided the blight-resistance gene, Rpi-vnt1.1. S. tuberosum cv. Desiree does not contain the blight-resistant gene. Therefore, the transferred Rpi-vnt1.1 gene is a foreign gene i.e. transgene.

This contradicts the description on the website cisgenesis.com of a cisgenic plant as one that contains "no foreign genes", which makes no sense when the reason they are putting in that gene is because the host plant does not have it! Most fundamentally, cisgenesis is still genetic engineering and employs the methods of transgenesis to make a GM crop with all the attendant risks. The new terminology is invented simply to deceive the public. The only difference is that the gene inserted may derive from an organism more closely related to the host species. This does not bypass any of the risks associated with standard GM procedures and as the transgene comes from a different species of potato, the protein product may indeed be different from the native non-transgenic potato. It should be recalled that the transfer of a gene between closely related species has led to

Categories

Cisgenesis/Intragenesis

- Stable integration of cisgene
- In the case of *Agrobacterium*-mediated transformation, presence of T-DNA border sequences
- Possible unintended effects:
  - Interruption of open reading frames (ORFs)
  - Creation of new ORFs
  - Deletion of host DNA
  - Genetransfer can lead to modified levels of gene expression

Cisgenesis/Intragenesis

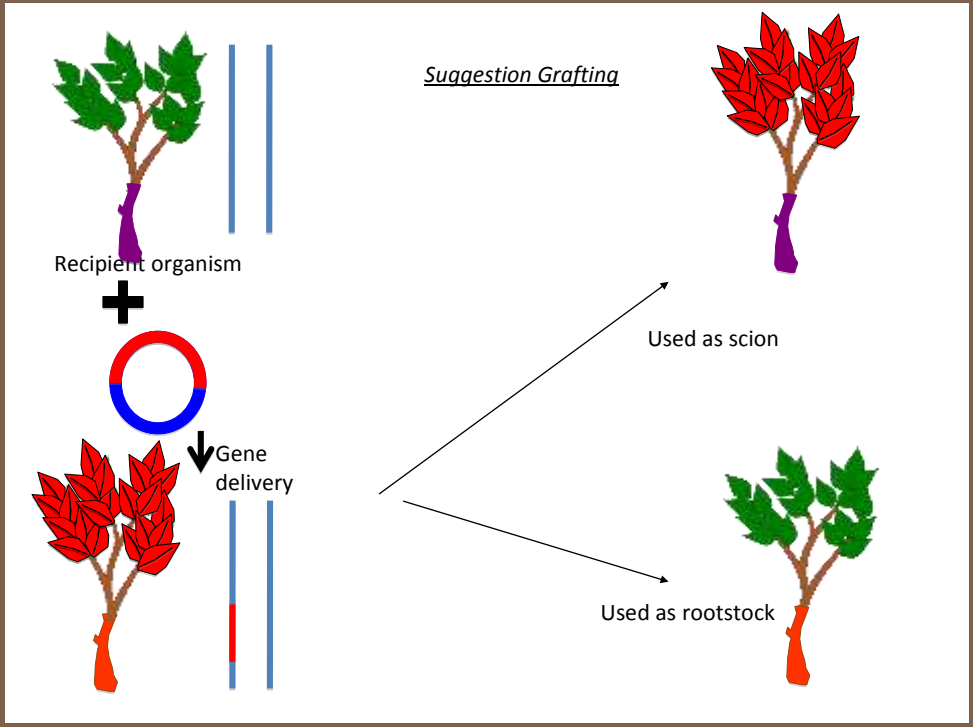
- Cisgenic/Intragenic plants can be detected and identified when adequate information is available
- Use of event-specific primers to differentiate from crops produced by conventional breeding
- In the case of unknown alterations, the detection and identification is currently not feasible
- Screening for cisgenic/transgenic plants is not possible due to absence of certain common elements (e.g. 35S promoter used in screening for unknown GMOs)

Source: "New plant breeding techniques: Challenges for detection and identification – report of the New Techniques Task force (NTTF)" in JRC Technical Report "New plant Breeding Techniques", EUR 24760 EN (2011)

Cepljenje:

GS rastlino lahko uporabimo kot podlago za cepljenje ali kot cepič.

Ali naj imamo rastlino, pri kateri je GS samo podlaga, za GSR ali ne?

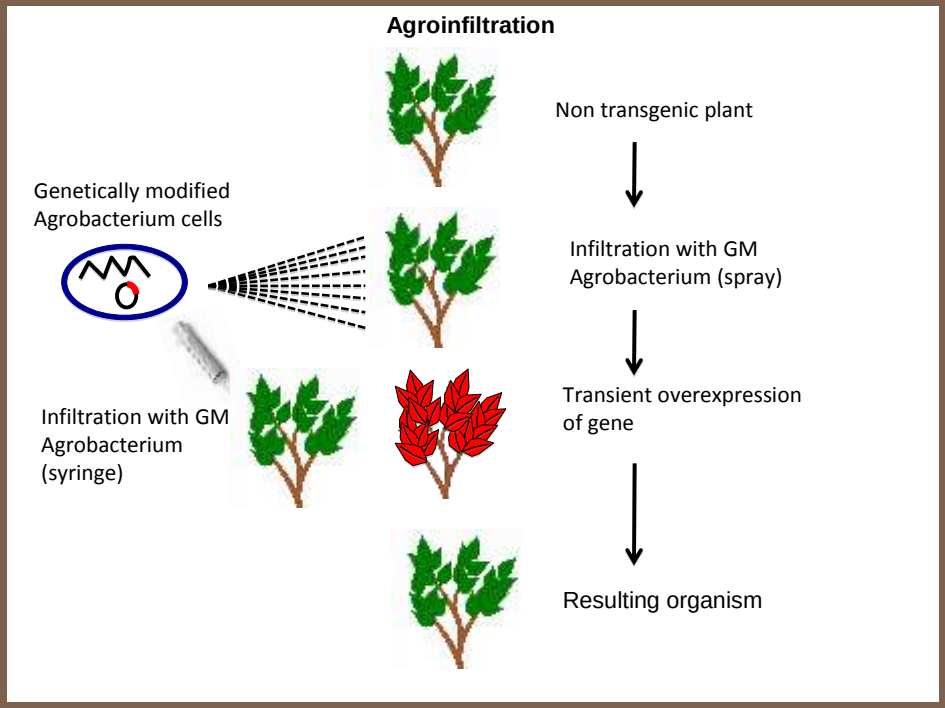


New Techniques Working Group - FINAL REPORT

Agroinfiltracija:

Transgen se le redko vključi stabilno v genom in tudi če se, ostaja le v nekaterih rastlinskih celicah. Cilj je, da rastlina izrazi nek gen in da preučimo fenotipske spremembe, ki so posledica prehodnega izražanja, ali pa, da rastlina proizvede nek rekombinantni protein, ki ga nato izoliramo.

Izvedba ‚floral dip' gensko spremeni tudi spolne celice in v tem primeru imamo za cilj pridobiti semena in GS rastline.



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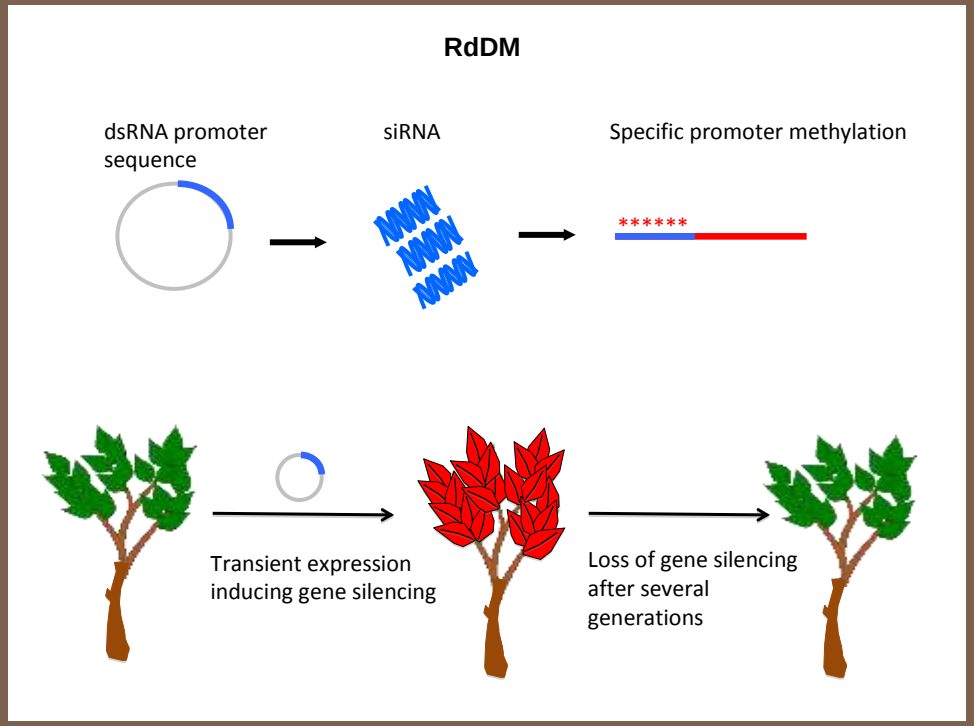
Od RNA odvisna metilacija DNA:

Izražanje siRNA v celicah lahko privede do metilacije DNA. Če je tarčna regija promotor, pride do utišanja gena. Epigenetska sprememba je prehodna in traja največ nekaj generacij.



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