UNIVERSITEIT GENT

FACULTY OF BIOSCIENCE ENGINEERING

Plant Breeding

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Hybrid breeding

- In **restricted sense** a hybrid is a cross between two genetically different (homozygous) inbred lines. Such a cross is also called a "F_1-hybrid" or a *single cross.*

- Products of a series of other crosses also may be called hybrids. In some plant species, it is not possible to create completely homozygous lines: due to inbreeding depression, they lose their fitness *after a few selfings*. Hybrids are then made by crossing lines that only have been selfed for a few generations. Examples: leek, cichory.

- In sugar beet e.g., usually only one of the crossing partners is an inbred line (completely or not completely homozygous); the pollinator is e.g. a population.

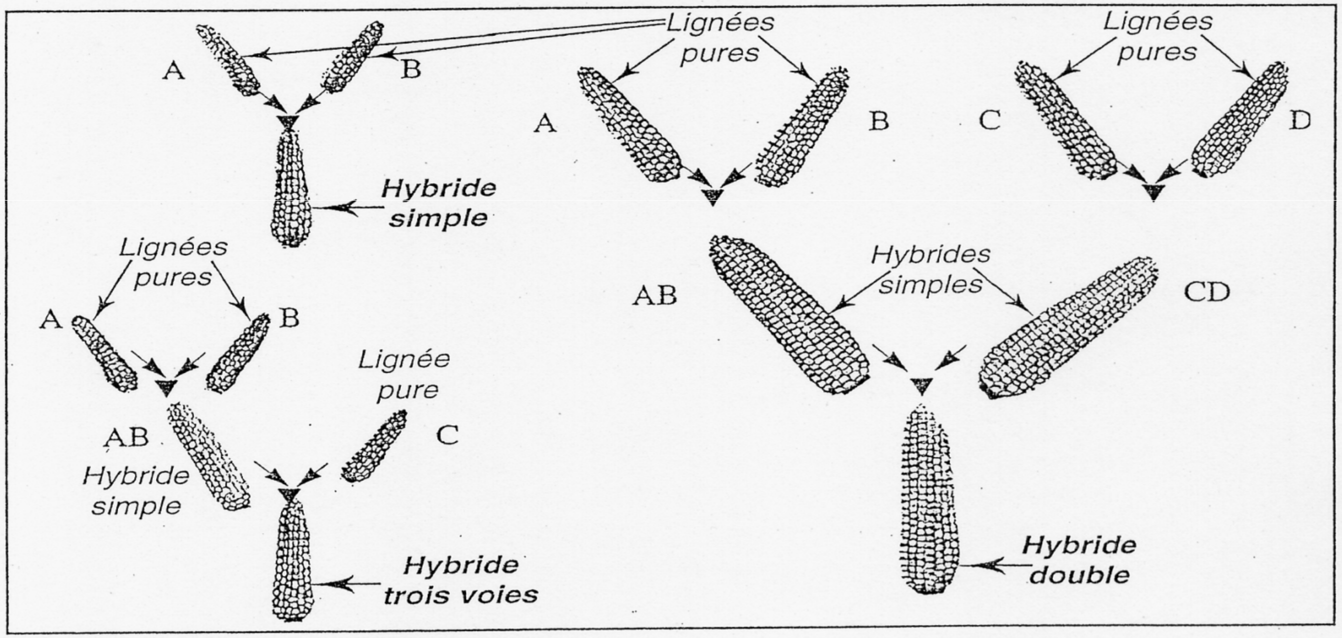
Dénominations	Hybride double HD				Hybride 3 voies HTV			Hybride simple HS	
Matériel de départ	Lignées				Lignées		Lignée L3	Lignée L1	Lignée L2
	L1	L2	L3	L4	L1	L2			
Semences de base	Hybride simple 1 L1 x L2		Hybride simple 2 L3 x L4		Hybride simple L1 x L2				
Semences certifiées	Hybride double (L1 x L2) x (L3 x L4)				Hybride trois voies (L1 x L2) x L3			Hybride simple L1 x L2	

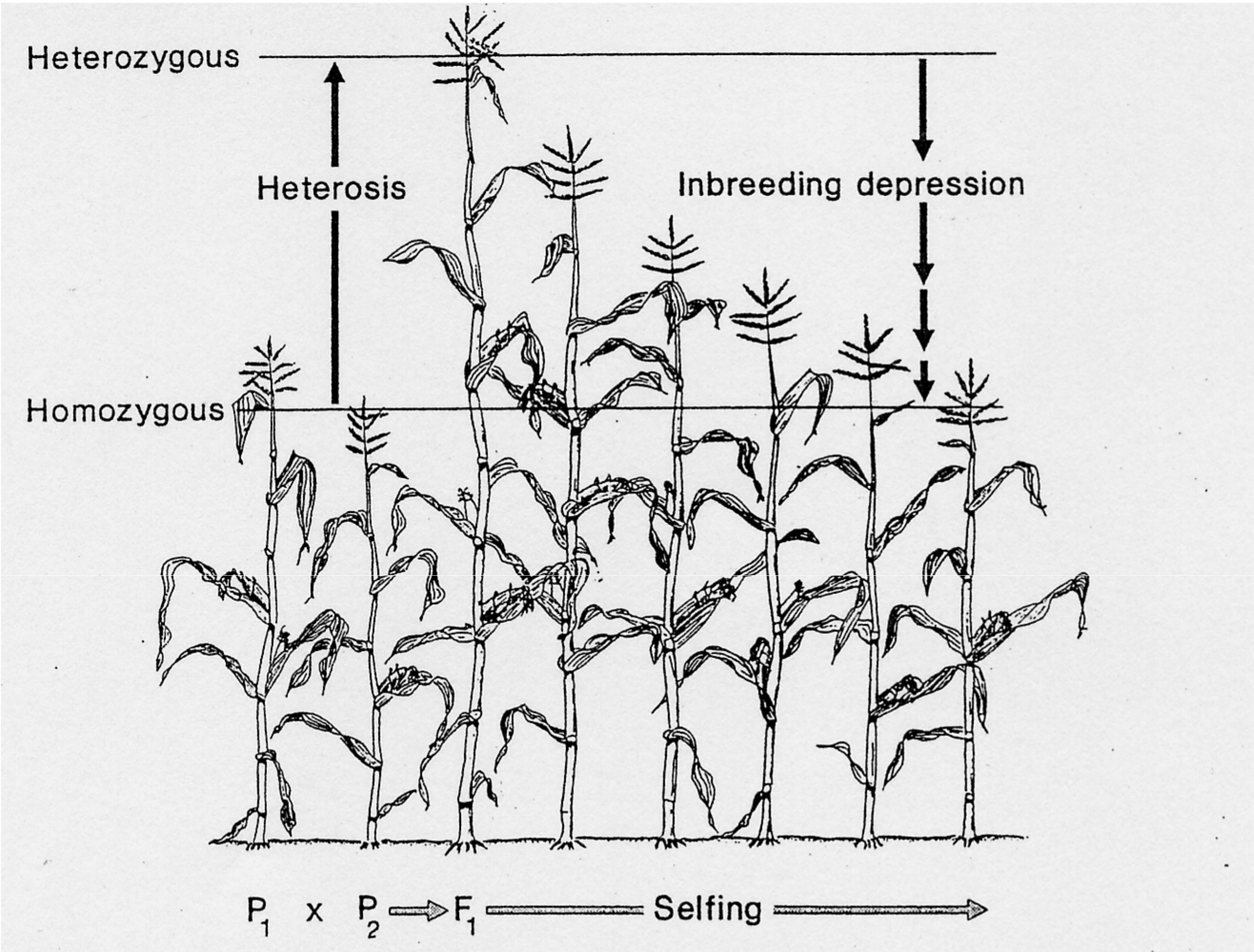
Figure 5-96 -

UN EXEMPLE :

LES TROIS TYPES D'HYBRIDES DE MAÎS

(Extrait du "Guide du producteur de semences certifiées de Maïs et de Sorgho", FNPSMS - 1996 Route de Pau, 64121 Montardon).




Heterozygous
Heterosis
Inbreeding depression
Homozygous
P1 x P2 F1 Selfing

Why hybrids ?

- Fixing the action of favourable genes in a cross-pollinator in a repeatable way (i.e. a cross-pollinator offers advantages of a self-pollinator…)
- Crop is vigourous: hybrid vigour, heterosis
- Crop is uniform ……
- Variety is biologically protected

Advantages | Disadvantages

For the plant breeder

Advantages

1. Reproducible combination of parental traits
2. Intrinsic variety protection
3. Growers need to buy new seeds every year

Disadvantages

Development of inbred lines, search for optimal combination, seed production and authenticity check is laborious and **costly**

For the plant grower

Advantages

Excellent VCU, stability and uniformity

Disadvantages

1. Expensive seed
2. Using farm saved seeds usually is not a good idea

The development of hybrid varieties in cross-pollinating crops without using male sterility case study: maize

Standard procedure

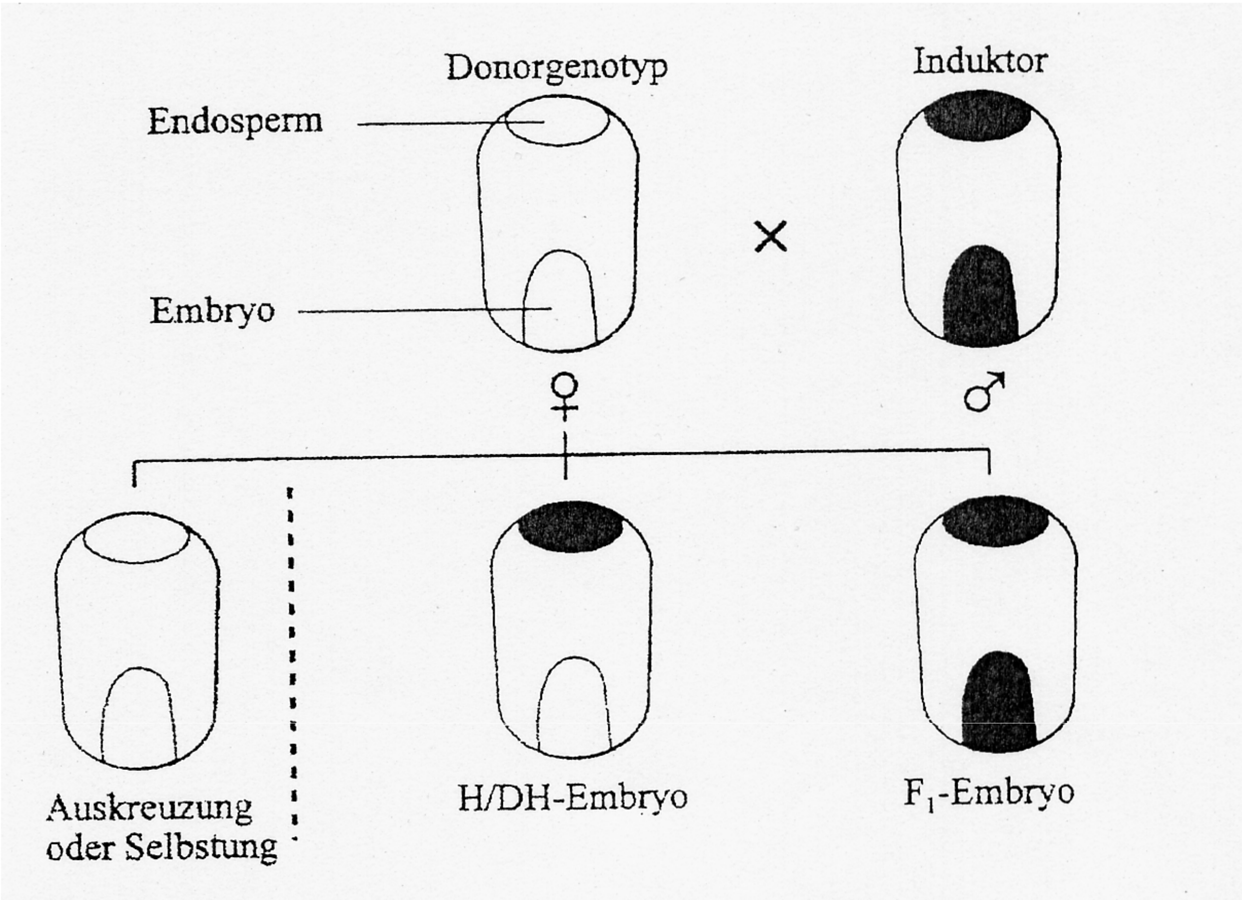
1. Development of inbred lines
2. Testing General Combining ability
3. Testing Specific Combining ability
4. Construction of the hybrid and hybrid seed production

The development of inbred lines in cross-pollinating crops without using male sterility case study: maize

a) Self polliniation
b) DH's…
c) Inducer line (Uni Hohenheim, Germany)….

When ?

Make S1's of F1's. Cross the superior S1's, F1's with the inducer line.



How the haploids or dihaploids in *in-vivo* induced haploids can be identified via xenia.

Röber, 1999.

Testing the combining ability

a) General combining ability (GCA) : testing the breeding value of the mother line using a genetically broad pollinator

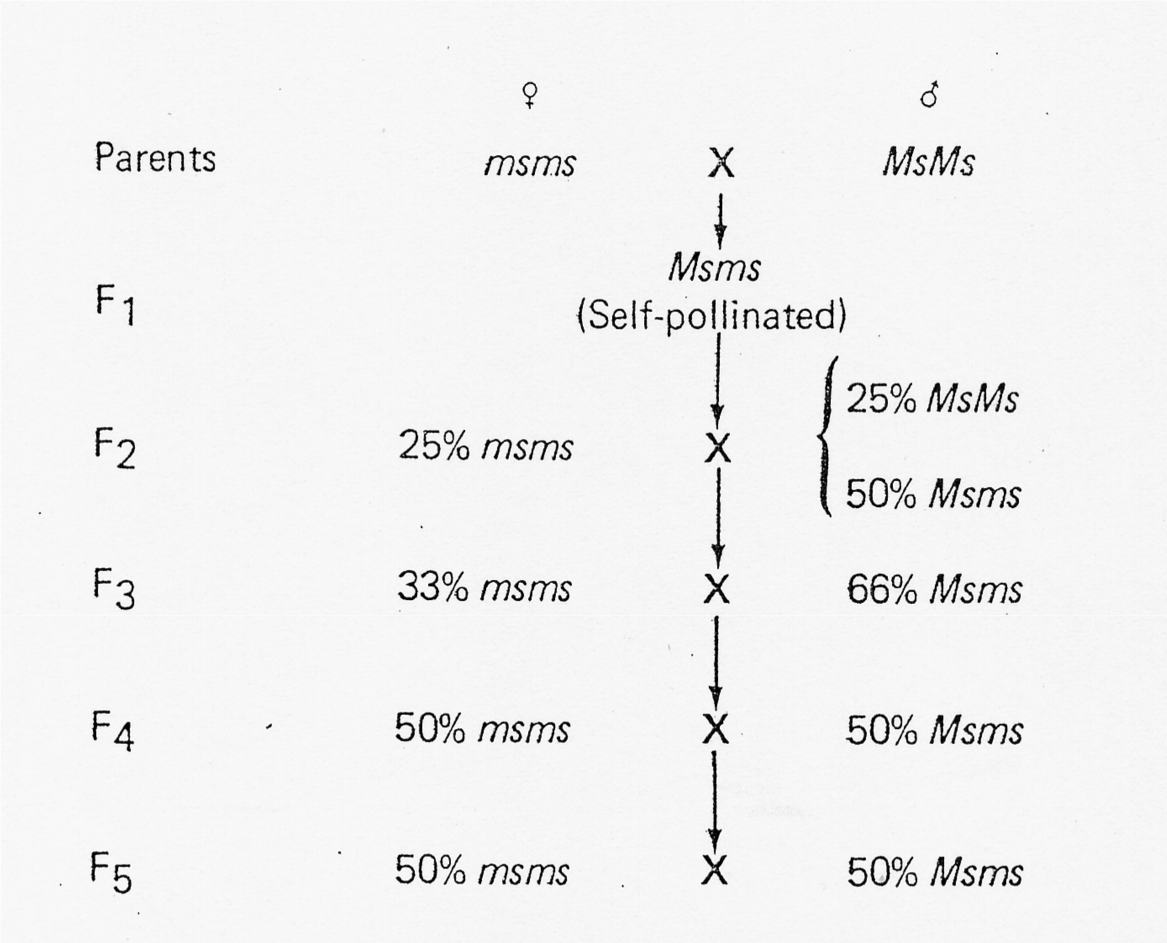
When ? Early testing

a) Specific combining ability (SCA): pair crosses

The development of hybrid varieties in cross-pollinating crops using male sterility

1. a) Genic (=nuclear male sterility): monogenetic recessive

   b) EGMS: monogenetic recessive
2. Transgenic male sterility: monogenetic dominant
3. CMS (cytoplasmatic male sterility): cytoplasm + recessive genes



Genic male sterility.

Proportions of male sterile (*msms*) and male fertile (*Msms* and *MsMs*) plants in case seeds are harvested on male-sterile plants only in the F_2 and succeeding generations.

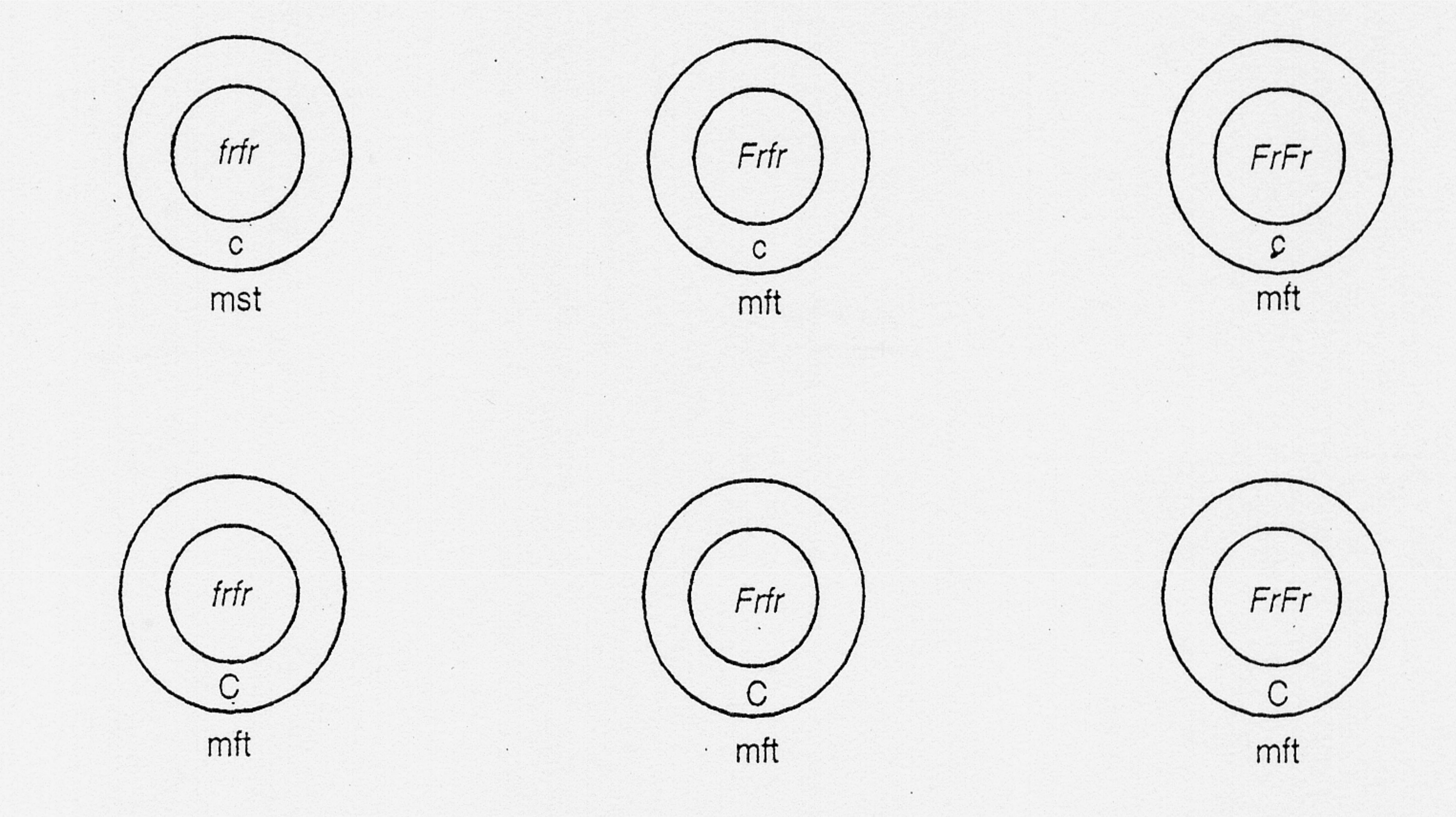
Poehlman and Sleper, 1995.

Transgenic male sterility

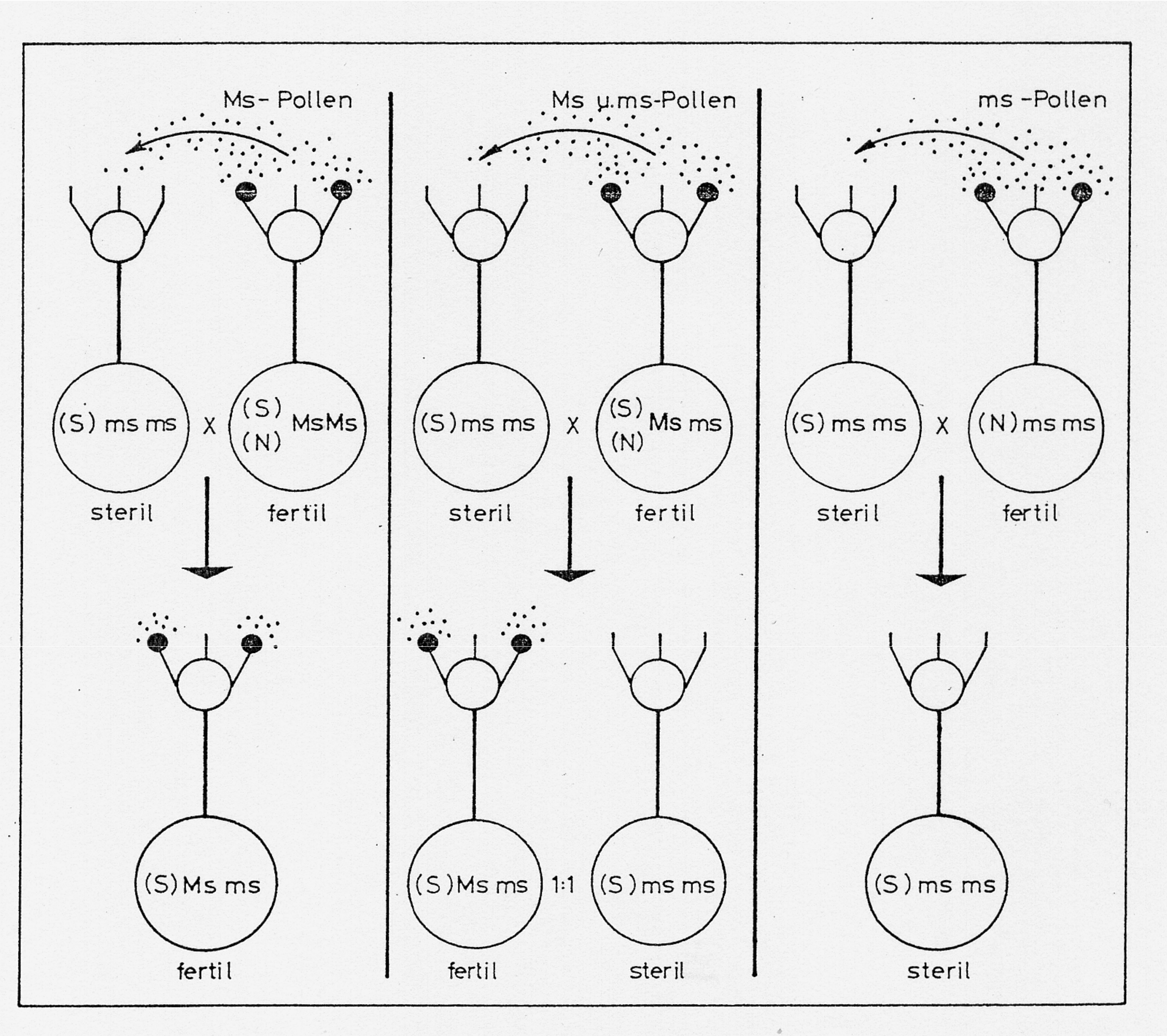
- Dominant inheritance
- Quite complicated and the use of restorer genes to develop a hybrid system is necessary
- Inserted genes ruin *tapetum* cells in pollen mother cells; hence pollen development is arrested
- Used in oilseed rape, ……..

CMS

- Combination of genes in mitochondria and *nucleus*
- Widely used in hybrid breeding



How CMS functions. Banga and Banga, 1998.



Maintainers (3th column) of male sterility in CMS.

Sneep and Hendriksen, 1979.

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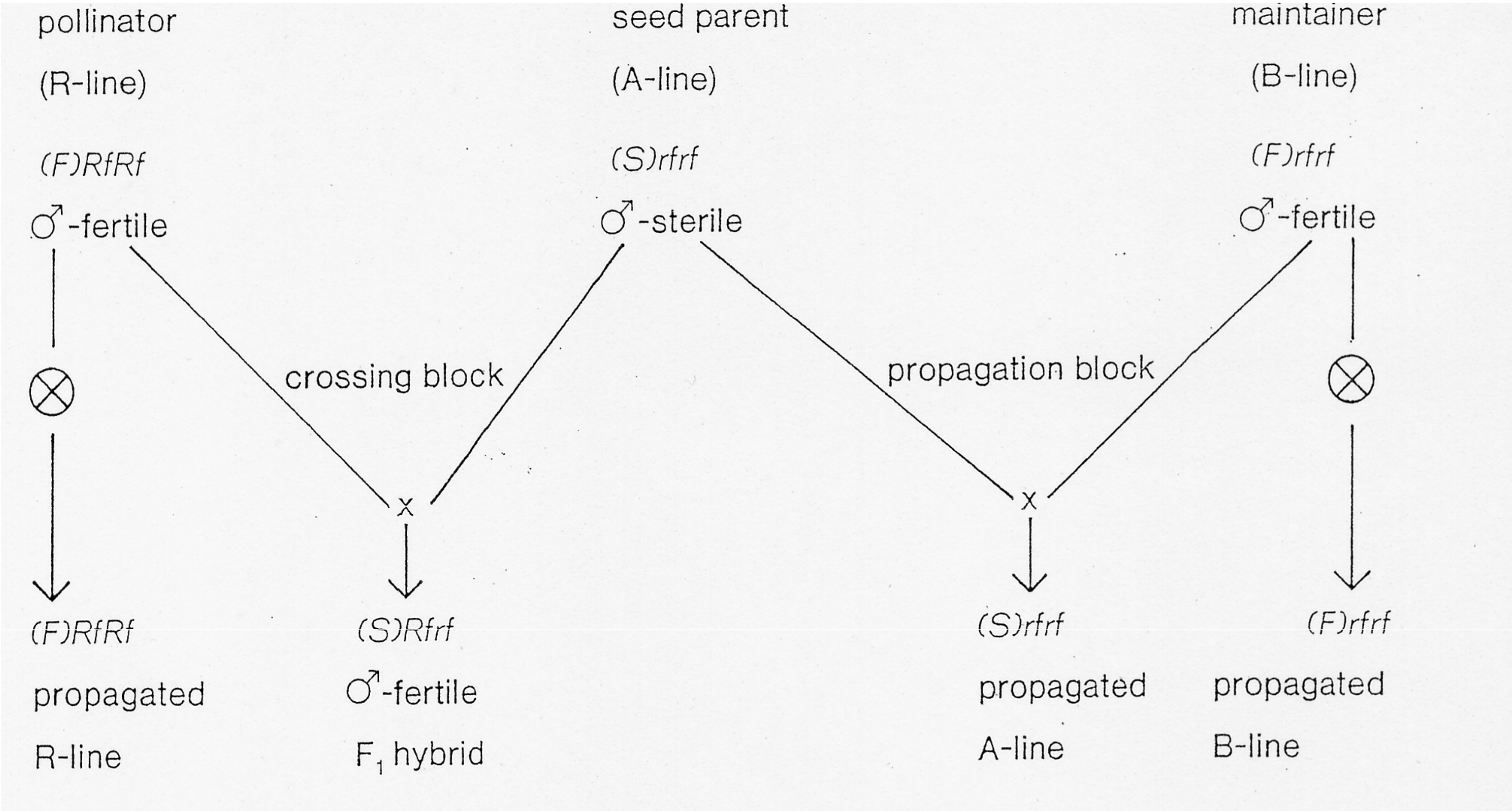
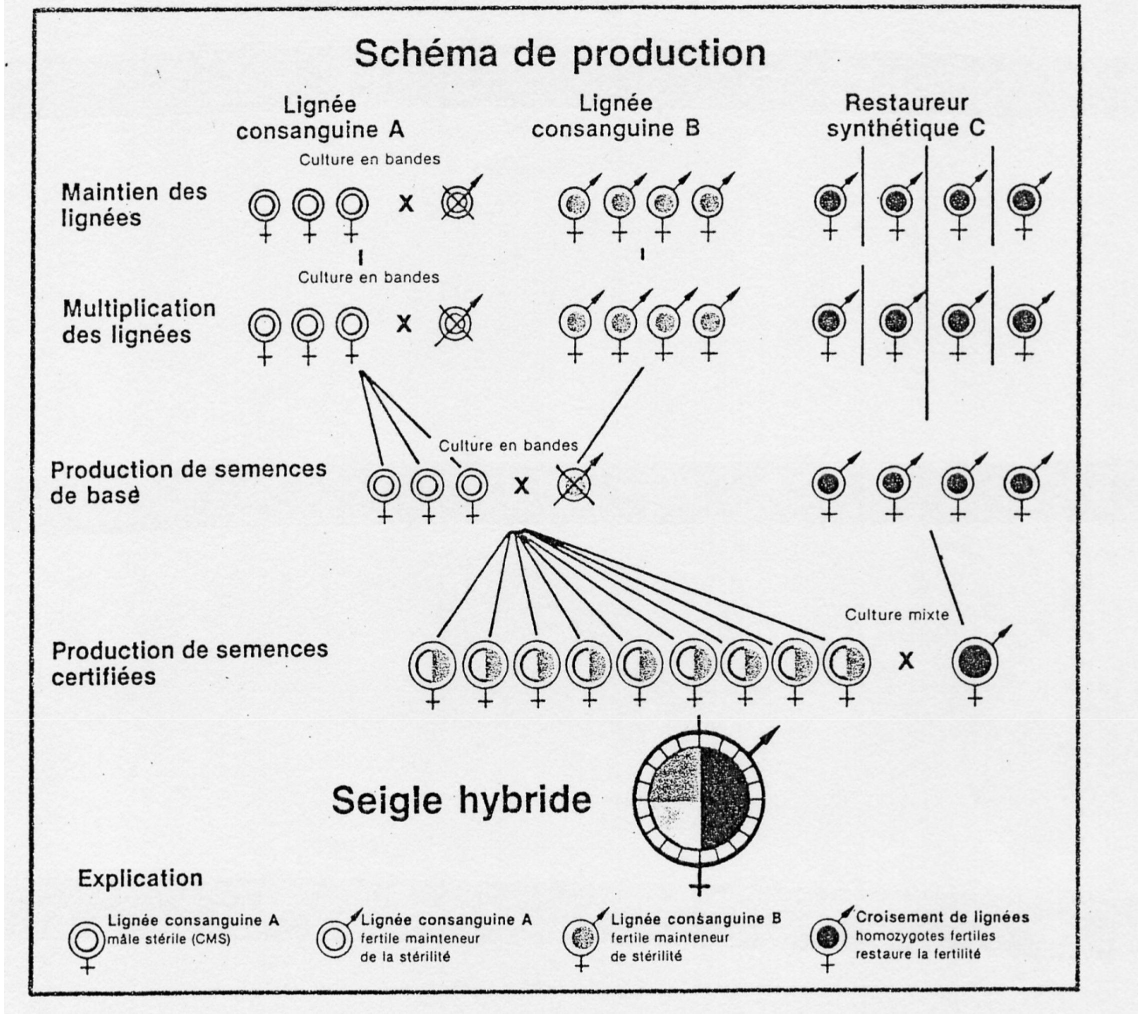


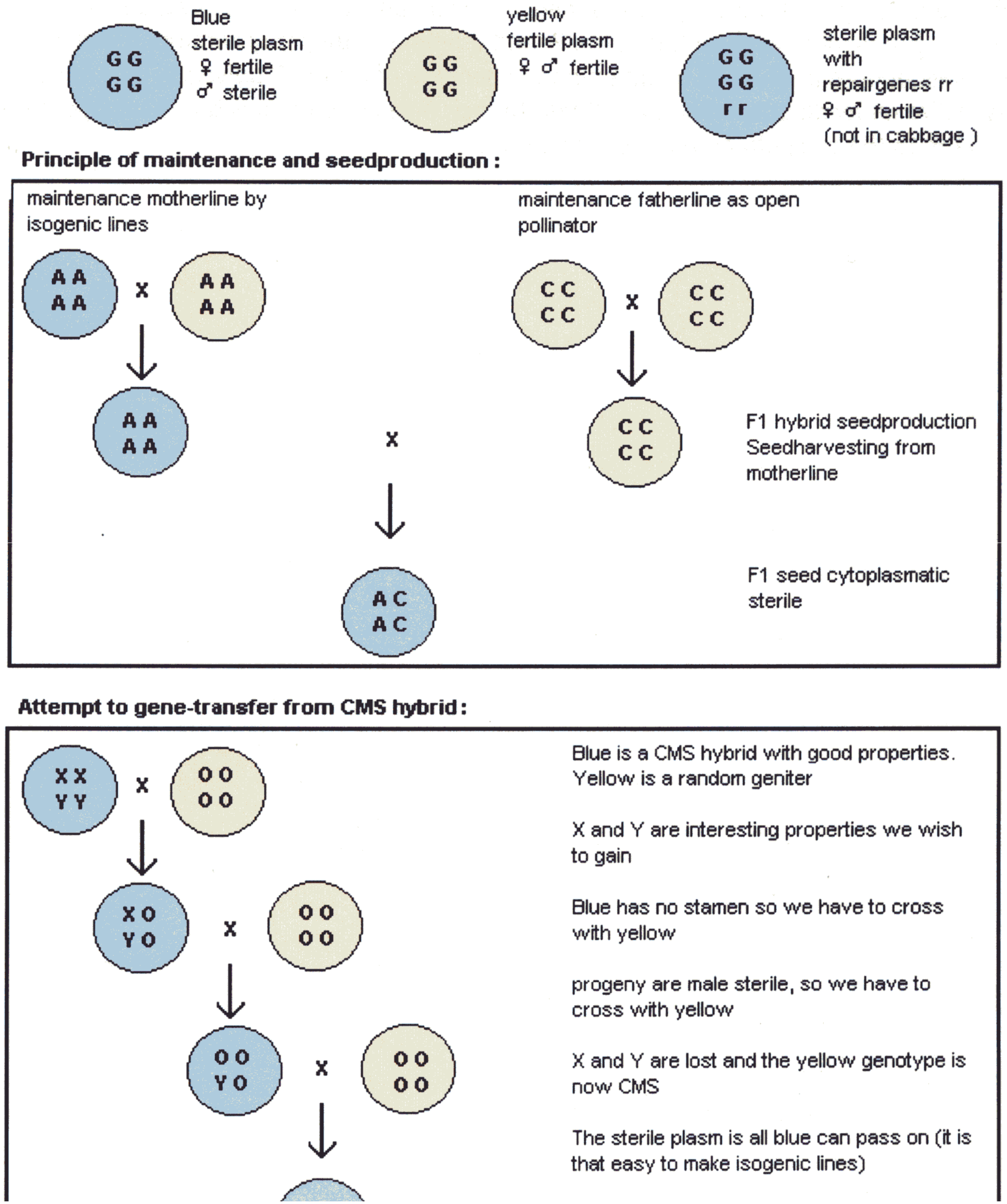
Hybrid production via CMS. Sneep and Hendriksen, 1979.



Hybrid seed producton in rye, using CMS.

De Felcourt et Deleplanque, 1988.

Cytoplasmatic Male Sterility – CMS



CMS with and without restorer genes.

Jan Velema, Vitalis Zaden, NL.

MAS to detect CMS sugar beet plants

Maintainers of (CMS) male sterility in sugar beet are called **'O-types',** named after the American Owen who published in 1945 about CMS and its maintainers in cultivar 'US-1'. O-types have a normal (fertile) cytoplasm and *rf* gene(s) in a homozygous recessive status. The cytoplasm of (CMS) male-sterile plants is called 'Owen cytoplasm'.

CMS male sterile plants are discovered upon inspection of flowers, which is a **laborious** task. O-types usually are detected by pair crosses: the offspring of a male sterile sugar beet pollinated by an O-type is male sterile again.

The Owen cytoplasm (CMS male-sterile plants) may be detected applying molecular markers. Indeed, the Owen cytoplasm has a new *Hind*III restriction site in the cpDNA (chloroplast DNA), strongly linked with a specific mitotype, characteristic for the Owen cytoplasm.

On top of this, variable number of tandem repeats (VNTRs) have been shown to provide a rapid and reliable procedure to distinguish normal cytoplasm and **different** sources of male-sterile cytoplasms from one another.

Among the many breeding problems under study at the All Union Sugar Beet Institute, Viacheslav (Savitsky) was very much interested in the development of a sugarbeet variety with single germed fruits instead of multigerm seedballs. The task of searching for plants with single-germed fruits was assigned to a junior staff member, M. G. Bordonos, who in turn had a scientific assistant, Mrs. O. K. Kolomiets, and her helpers do much of the searching. In a paper published in 1960, **Bordonos reports that more than 22 million seedbushes (1023 hectares) were examined in 1934 with 109 plants producing monogerm fruits in addition to the usual multigerm seedballs.**

JS McFarlane, Journal of Sugar Beet Research, Jan-June 1993

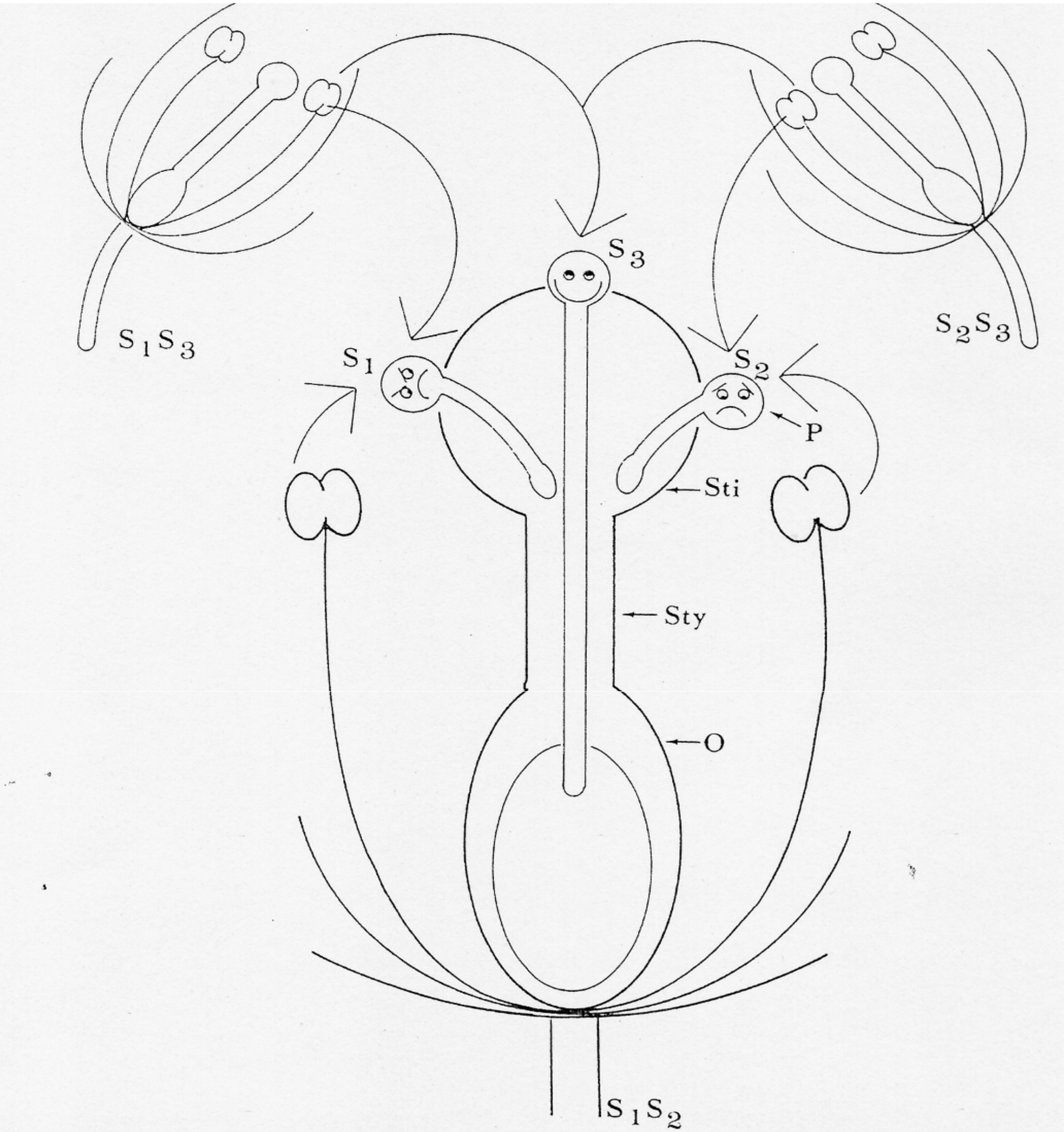
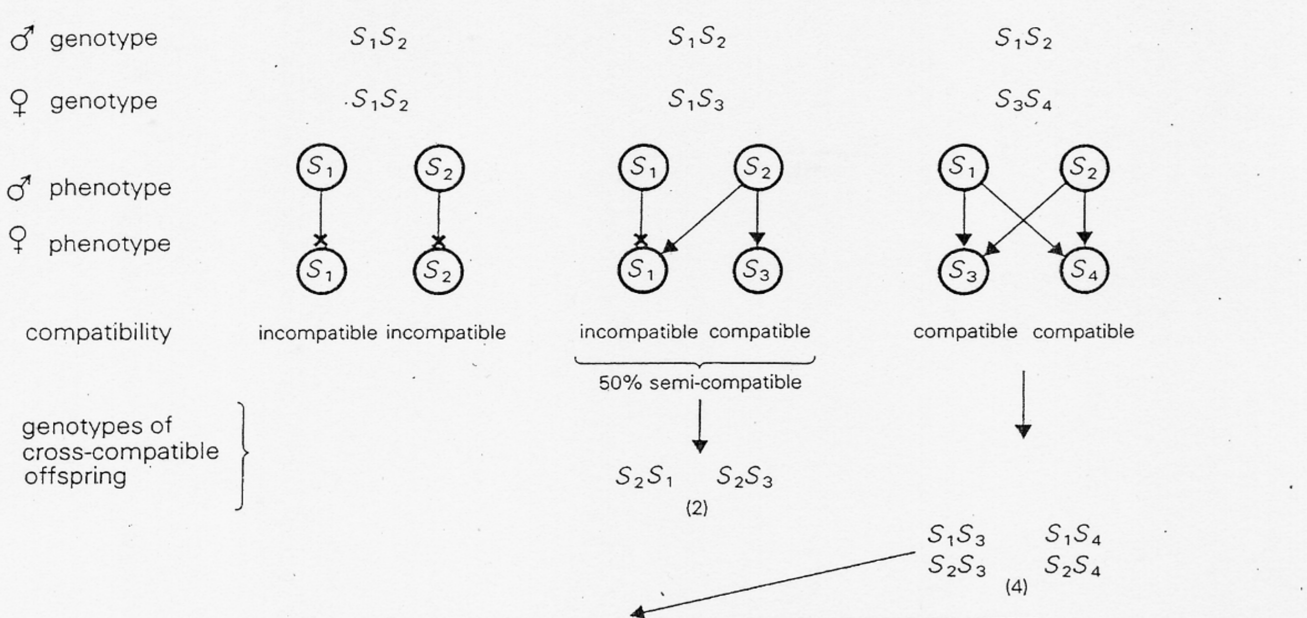


FIG. 2 Control of gametophytic self-incompatibility (GSI) by the multiallelic S locus. The figure shows the outcome of pollen–pistil interactions between a flower of S_1S_2 genotype (female parent) and pollen from the same flower or flowers of S_1S_3 or S_2S_3 genotype. Pollen that bears the S_1 or S_2 allele is incompatible on the S_1S_2 pistil. Pollen bearing the S_3 allele is compatible because it does not share a common S allele with the recipient pistil. Compatible pollen is able to grow into the ovary and effect fertilization, whereas the growth of incompatible pollen is arrested in the style. O, ovary; Sty, style; Sti, stigma; P, pollen.

Gametophytic 1 locus system of self-incompatibility.

Singh and Kao, 1992.

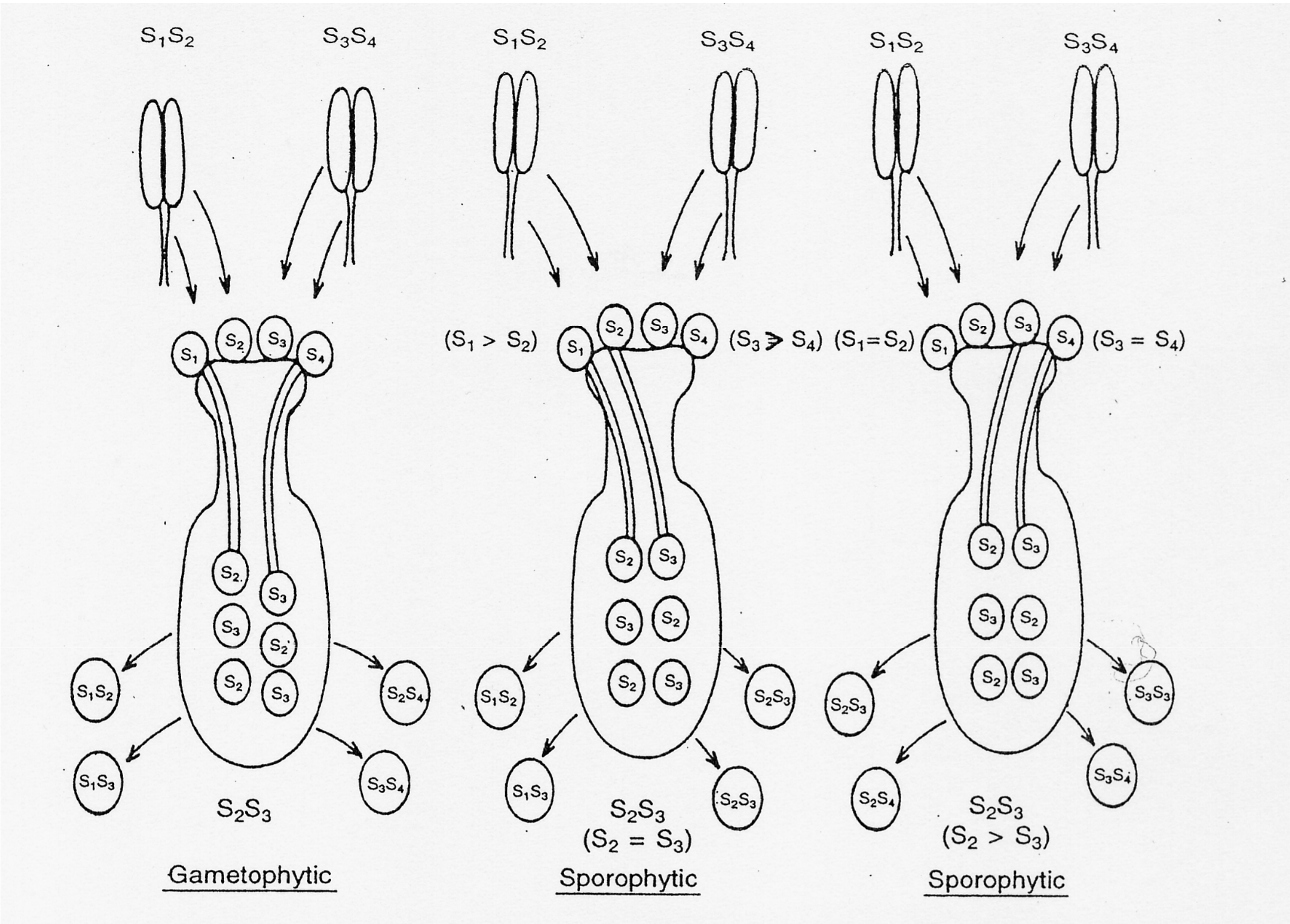


	S_1S_3	S_1S_4	S_2S_3	S_2S_4
S_1S_3	incompatible	semi-compatible	semi-compatible	compatible
S_1S_4	semi-compatible	incompatible	compatible	semi-compatible
S_2S_3	semi-compatible	compatible	incompatible	semi-compatible
S_2S_4	compatible	semi-compatible	semi-compatible	incompatible

for any one offspring, 25% of the others will be cross-incompatible (share the same genotype), 50% will be semi-compatible, and 25% fully compatible; there will be four distinct sibling genotypes from a single cross

Behaviour and inheritance of S alleles in one-locus gametophytic self-incompatibility.

Richards, 1997.



Difference between sporophytic and gametophytic s.i.

The mother plant is S_2S_3. Pollinators are S_1S_2 and S_3S_4 .

'>' indicates dominance.

'=' indicates co-dominance.

Banga and Banga, 1998.

Hybrids via self-incompatibility (SIC)

Gametophytic SIC: hybrids via vegetative propagation of parents

Sporophytic SIC: lines homozygous for S-alleles

Inbred line A	$\mathbf{S_1S_1}$	**x**	$\mathbf{S_2S_2}$	**Inbred line B**
Hybrid		$\mathbf{S_1S_2}$		

Inbred line A S_1S_1 x S_2S_2 S_3S_3 x S_4S_4 **Inbred line B**

S_1S_2 x S_3S_4

Hybrids S_1S_3, S_1S_4, S_2S_3, S_2S_4

Development of hybrid varieties in self-pollinating crops

Hand emasculation: tomato

CHA’s: chemical hybridization agents; gametocides
wheat

Development of hybrid varieties in self-pollinating crops: EGMS in rice

- In TGMS, plants are male sterile at high temperatures
- In PGMS at a long daylength
- In case the plants are growing at low temperatures/ short daylengths, they are completely fertile.
- **Inheritance is monogenous recessive.**

CMS is a 3 line sytem, EGMS a 2 line system

- Maintainers are no longer necessary, since “the environment” acts as a maintainer.
- Hybrids can be produced with 2 instead of 3 lines ! Seed parents (which should be male sterile in hybrid seed production fields) are transferred to an appropiate environment (where they become fertile) in order to multiply them. The system works very well in a large country with different climates and altitudes.
- Because one has not to take care of maintainers, one can test more combinations (male sterile x pollinator) hence increasing the probability to discover a top combination.

Hybrids in dioecious plants : Asparagus

Asparagus (*Asparagus officinalis* L.) is a dioecious plant. Male plants produce heavier stems than female plants and are less susceptible to diseases and pests. Female plants produce seed: the berries fall down and during the next growing season volunteers (seedlings) must be removed (mechanically or with herbicides).
Sex is controlled by nuclear genes in the *M*-locus. Male plants are *Mm*, female plants *mm*; the *M* allele being dominant. In spontaneously occurring populations half of the plants are male and half of the plants are female: indeed female plants produce *m* gametes, male plants both *M* and *m* gametes in a 1:1 rate.

Gametes and *zygotes*

Male plant = Mm; Female = mm

♂ / ♀	**M**	**m**
m	***M**m*	***m**m*

Hybrids in dioecious plants : *Asparagus*

Andromonoecious plants occur in very low frequencies. *MM* males may result from self-pollination of their perfect flowers. Phenotypically these “supermales” are undistinguishable form *Mm* males. They must be identified by testcrosses.

In order to produce a pure male variety, the pollinator producing the commercial seed must be a **supermale**: indeed its offspring is all *Mm*.
How do breeders get supermales ? Via pollen cultures. Chromosome doubling results in **homozygous** diploid fertile *mm* or *MM* plants.

Supermale seedlings are propagated *in vitro* to get high numbers of pollinators. Pollinators are planted in the hybrid seed production field next to propagated females. The harvested hybrid seed produces exclusively male plants that are planted in the fields of *Asparagus* growers.

In 2009, Alexa Telgmann-Rauber found **molecular markers** that are closely linked to the *M*-locus: 3 female specific markers and 12 male specific markers, some of them being co-dominant, offering the opportunity to distinguish supermales from heterozygous male plants, hence eliminating the need of testcrosses to identify supermales.

Reverse breeding

1. In hybrid breeding, inbred lines are developed from superior plants.
Via GCA and SCA tests the heterozygous individual with the highest heterosis is identified. This plant probably does not occur spontaneously in a population: it is an "artificial" combination.

Crossing the multiplied inbred parents allows the production of high quantities of commercial hybrid seed in a repeatable way.

2. Superior plants are always present in populations of cross-pollinators: genetically they are "by chance" combinations of matching genes and alleles Unfortunately their genotypes are crumbling in the following generations due to segregation and recombination during meiosis.

If one could re-synthesize these superior combinations in a repeatable way, one would have found:
(1) a way to fix elite (spontaneous) gene combinations
(2) an alternative way to exploit the within crop genetic variability.

Reverse breeding

The idea of **reverse breeding** is this.
If one could stop recombination during meiosis (by down regulation of the responsible genes by RNAi) in an elite plant, all its gametes would contain different combinations of unchanged chromosomes of the father and mother of the elite plant.

The production of doubled haploids would (quickly) produce fertile homozygous plants.

By studying the origin of the chromosomes in each DH, one would be able to identify 2 DH's which, upon crossing, would resynthesize the genotype of the original elite plant.

A patent application has been filed by the Dutch Rijk Zwaan vegetable seed company.

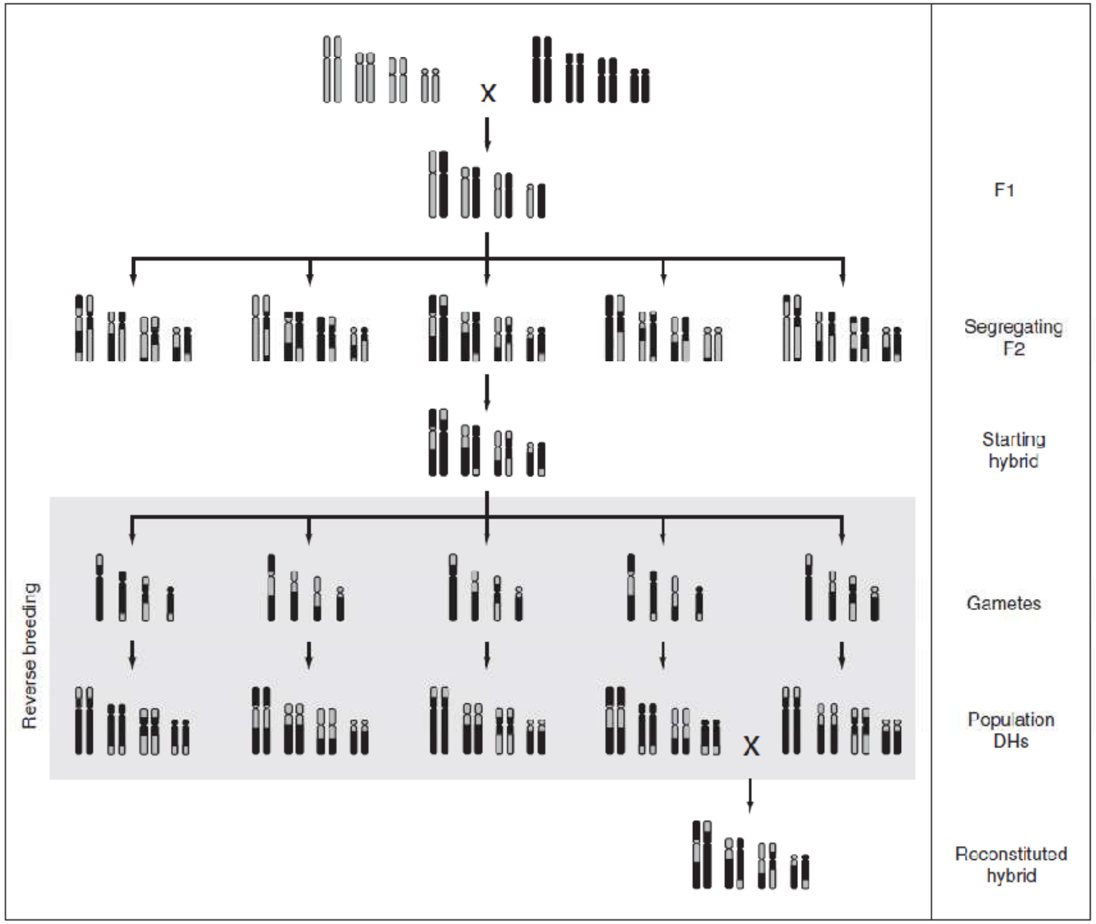


Figure 1 Reverse breeding can be used to fix unknown heterozygotes. Crossing two homozygous parents (grey and black bars) creates a heterozygous F1. When selfed, the F1 produces a segregating F2 population. A starting hybrid of unknown genetic constitution is selected for its desireable characteristics, and subjected to the two steps of reverse breeding (grey box). By knocking down meiotic crossing over, whole parental chromosomes are transmitted through spores, without rearrangement. Note, in this example the four chromosomes in the hybrid can generate 16 different combinations in the gametes—only five are shown for convenience. The achiasmatic gametes are then used produce doubled haploid (DH) lines using *in vitro* culture techniques. From this population, complementary parents can be chosen that when crossed perfectly reconstitute the starting hybrid. The DH lines then serve as a permanent library that can be used to predictably generate a wide variety of defined hybrids.

Dirks et al. 2009, Plant Biotechnology Journal