SISTEMI OPRAŠEVANJA

Poznavanje sistema opraševanja je pomembno zaradi več razlogov:

i) Genetska struktura rastline je odvisna od načina razmnoževanja (če se le da, se genetsko strukturo ohrani, v nasprotnem primeru je vzdrževanje sorte zahtevnejše)

ii) Za proučevanje dedovanja lastnosti je potrebno izvesti načrtna križanja, zato je poznavanje biologije razmnoževanja določene rastlinske vrste nujno.

iii) Pri hibridizaciji je ključno zagotoviti oprašitev z želenim pelodom. Nadzor oz. kontrola opraševanja je ključna pri pridobivanju hibridnega semena.

iv) Način razmnoževanja določa postopke za razvoj in ohranjanje kultivarjev.

1

Dvospolni (hermafroditni) cvetovi / enospolni

Samoopraševanje (samoprašnice, avtogamne rastline) / navzkrižno opraševanje (tujeprašnice, alogamne rastline)

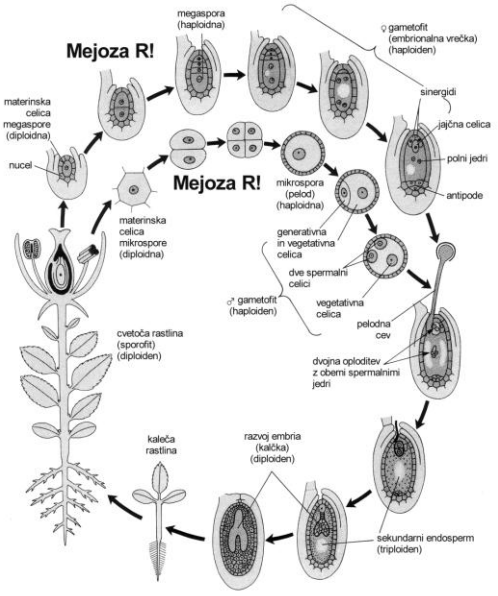
Spolno razmnoževanje / nespolno (aseksualno) razmnoževanje (vegetativno (brez semena), agamospermija (apomiksis, razvoj semen brez oploditve)

Delitev rastlin glede na dolžino življenskega cikla: enoletnice (ozimne, letne), dvoletnice (zelje, sladkorna pesa), trajnice.

Monoecične / diecične rastline

2

Življenjski krog pri kritosemenkah



Batič F., Šircelj H. in Turk B. 2004. Pregled rastlinskega sistema. Biotehniška fakulteta, Ljubljana.

3

Razvoj ženskega gametofita

Praspolna (arhespolna) celica preide v stadij diploidne materinske celice makrospore. Po mejotičnih delitvah nastanejo štiri haploidne makrospore od katerih tri propadejo, iz preostale makrospore se razvije **zarodkov mešiček oz. ženski gametofit**. Prvotno eno jedro materinske celice zarodkovega mešička trikrat zaporedoma deli na dva, štiri in osem jeder. Iz treh jeder se razvijejo na mikropilarnem polu jajčna celica in dve sinergidi, ki skupaj tvorijo jajčni aparat. Na nasprotnem polu nastanejo tri celice antipode. Preostali dve jedri pa odpotujeta na sredino zarodkovega mešička. Antipode imajo prehranjevalno funkcijo, sinergidi pa poleg prehranjevalne še encimsko funkcijo. (mešiček 8 jederni, 6 celični)

Embrionalni razvoj prašnikov, pelodnih vrečk in pelodnih zrn

Arhespolne celice v pelodnih vrečkah se mejotično delijo, najprej nastane celična diada, nato pa tetrada pelodnih zrn. Proces nastanka mikrospor ali pelodnih zrn se imenuje mikrosporogeneza.

4

Razvoj moškega gametofita
Mikrospora - pelodno zrno je v začetku vedno enojedrno, nato se deli na večjo vegetativno (ki propade) in manjšo generativno celico in v tem stanju poteka oprašitev. Ko pade pelodno zrno na brazdo pestiča, kali tako, da zraste intima v pelodno cev. Generativna celica se predeli v dve spermalni celici. Takšno kaleče pelodno zrno predstavlja **moški gametofit**.

Oploditev

Ko pelodna cev prodre do semenskih zasnov, se pod vplivom encimov sinergid odpreta zarodkov mešiček in pelodna cev, nakar pride do dvojne oploditve. Ena spermalna celica se združi z jajčno celico v oplojeno jajčno celico zigoto (2n), druga spermalna celica se združi z dvemi jedri zarodkovega mešička v triploidno celico, iz katere nastane triploidni sekundarni endosperm (hranilno tkivo).

V povprečju je čas od oprašitve do oploditve pri češnji 3-5 dni, pri slivi 7-8, pri jablani 7, pri leski 2-3 mesece.

5

Primeri samoprašnic		Primeri tujeprašnic	
ječmen	*Hordeum vulgare*	banana	*Musa sp.*
čičerika	*Cicer arietinum*	zelje	*Brassica oleracea*
fižol	*Phaseolus vulgaris*	korenje	*Daucus carota*
bombaž	*Gossypium spp.*	kasava	*Manihot esculentum*
jajčevec	*Solanum melongena*	kumare	*Cucumis sativa*
lan	*Linum usitatissimum*	koruza	*Zea mays*
solata	*Letuca sativa*	čebula	*Allium spp.*
oves	*Avena sativa*	krompir	*Solanum tuberosum*
grah	*Pisum sativum*	redkev	*Raphanus sativus*
breskev	*Prunus persica*	rž	*Secale cereale*
riž	*Oryza sativa*	sladkorna pesa	*Beta vulgaris*
sirek	*Sorghum bicolor*	sončnica	*Helianthus annuus*
soja	*Glycine max*	sladki krompir	*Ipomoea batatus*
tobak	*Nicotiana tabacum*	lubenica	*Citrullus lanatas*
paradižnik	*Solanum lycopersicum*	repa	*Brassica compestris*
pšenica	*Triticum aestivum*	česen	*Allium sativum*

6

Mehanizmi, ki vplivajo na način opraševanja

Mehanizmi, ki povečujejo delež samooprašitve

Kleistogamija

Mehanizmi, ki preprečujejo samooprašitev/povečujejo možnost navzkrižnega opraševanja

Zgradba cveta:
- diecične rastline – konoplja, hmelj, šparglji, špinača, sladkorna pesa;
- monoecične rastline - koruza

Dihogamija: protandrija / protoginija

Samo-inkompatibilnost

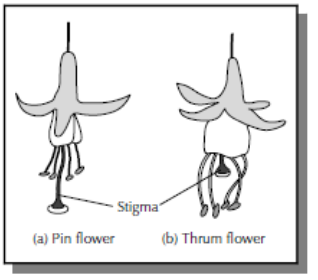
7

Samo-inkompatibilnost: heteromorfna in …

Heterostilija: razlike v dolžini prašnikov in pestiča
(primer sporofitne inkompatibilnosti)

- Pin tip: prašniki so krajši od pestiča
- Thrum tip: pestič krajši od prašnikov (primer: *Primula*)

Ss (thrum) in ss (pin) rastline
Kompatibilne rastline le pin x thrum (ss x Ss)
Križanja med morfološko enakimi cvetovi so sterilna.



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… in homomorfna (gametofitna, sporofitna)

1) GAMETOFITNA INKOMPATIBILNOST

Inkompatibilnost določa haploidni genom gametofita (peloda) – in ne genom staršev – pogojujejo jo S aleli. S aleli ne kažejo dominance in vsak deluje individualno pri regulaciji reakcije med brazdo in pelodom. Pestič je diploidno sporofitno tkivo in ima zato dva S alela, običajno v heterozigotnem stanju, medtem ko je v pelodu le en S alel. Opraševanje je kompatibilno takrat, ko je S alel peloda različen od S alelov materne rastline. V primeru gametofitne inkompatibilnost se generativno jedro deli šele po kalitvi pelodne cevke. Zigota je heterozigotna za S alel. Mesto inhibicije v tkivu pestiča.
Pojavlja se v družinah Poaceae, Solanaceae, Rosaceae, itd.

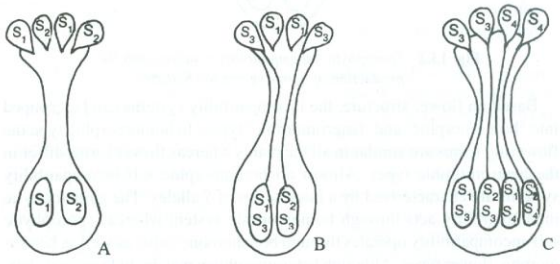


Fig. 13.1 *Gametophytic incompatibility resulting into the production of only heterozygotes for S alleles*

Chal & Gosal (2002) Principles and Procedures of Plant Breeding

9

Determination of self-incompatible genotypes in sweet cherry (*Prunus avium L.*) accessions and cultivars of the German Fruit Gene Bank and from private collections

M. Schuster, H. Flachowsky, D. Köhler

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Abstract

Sweet cherries are self-incompatible because of a gametophytic self-incompatibility system. *S* alleles in the style and pollen determine the crossing relationships. Knowledge of the *S* allele constitution of cultivars is very important for cherry growers and breeders, and recently, molecular methods have been developed to distinguish the *S* alleles in sweet cherries. The *S* allele genotypes of 149 sweet cherry cultivars and clones, including 126 not previously genotyped, were determined by using PCR analysis. Thirteen different *S* alleles in 40 combinations were distinguished and nine new incompatibility groups were documented. Two new *S* alleles were identified in five local sweet cherry processing cultivars from southwestern Germany using the second intron primers. The sequence of these alleles was determined and compared to all known sequences available in the NCBI database. The sequences obtained showed high similarities to the alleles S_{19} and S_{22}, previously described only in wild cherries, *Prunus avium* L.

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Table 2: *S* genotypes of 149 sweet cherry cultivars/clones

		Results with consensus primers													
		Second intron		First intron		Amplification with allele-specific primers**									
Incompatibility group	Origin§	Allele 1	Allele 2	Allele 1	Allele 2	S_1	S_2	S_3	S_5	S_7	S_9	S_{12}	S_{13}	S_{14}	S_{22}
Group I S_1S_2															
Rivan	RU	S_1	S_2	S_1	S_2										
Starking Hardy Giant[9,10]	US	S_1	S_2	S_1	S_2	+	+								
Group II S_1S_3															
Braune Prinzer	DE	S_1	S_3	S_1	S_3										
clone 11W26-58	CA	S_1	S_3			+		+	–						
clone 92/23-9	DE	S_1	S_3	S_1	S_3	+			–						
Giant Red	US	S_1	S_3	S_1	S_3										
Heidi	DE	S_1	S_3	S_1	S_3	+		+							
Huldra	SE	S_1	S_3	S_1	S_3										
Ladeho Pozdni	CZ	S_1	S_3	S_1	S_3	+			–						
Merton Bigarreau	GB	S_1	S_3	S_1	S_3										
Samba[5,10]	CA	S_1	S_3	S_1	S_3										
Schubacks Frühe	DE	S_1	S_3	S_1	S_3	+		+							
Sumbigo	CA	S_1	S_3	S_1	S_3										
Sumbola	CA	S_1	S_3	S_1	S_3										
Sweet September	US	S_1	S_3	S_1	S_3										
Group III S_3S_4															
Altenburger Melonenk[2]	DE	S_3	S_4												
Didi	DE‡	S_3	S_4			–									
Große Prinzess	DE	S_3	S_4	S_3	S_4			+							
Karina	DE	S_3	S_4	S_3	S_4			+							
Langstieler (Wiegele)	DE‡	S_3	S_4					+							
Pflugwirtle (Huber)	DE‡	S_3	S_4												
Solymari Scinscerznye	HU	S_3	S_4												
Somerset[5,10]	US	S_3	S_4												
Teickners Schwarze Herzk	DE	S_3	S_4	S_3	S_4			+							
Transportabelnaja	UA	S_3	S_4	S_3	S_4			+							
Ulrichs Braune	DE	S_3	S_4	S_3	S_4			+							
Werdersche Braune	DE	S_3	S_4	S_3	S_4			+							
Group IV S_2S_3															
Blankenburg	DE	S_2	S_3												
Frühreste der Mark	DE	S_2	S_3	S_2	S_3		+	+							
Kassins Fruhe[2]	DE	S_2	S_3	S_2	S_3										
Pollux	NL	S_2	S_3												
Group V S_4S_5															
Althof	DE‡	S_4	S_5						+						
Maisenbühler	DE‡	S_4	S_5						+						
Schwarze	DE‡	S_4	S_5						+						

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… in homomorfna (gametofitna, sporofitna)

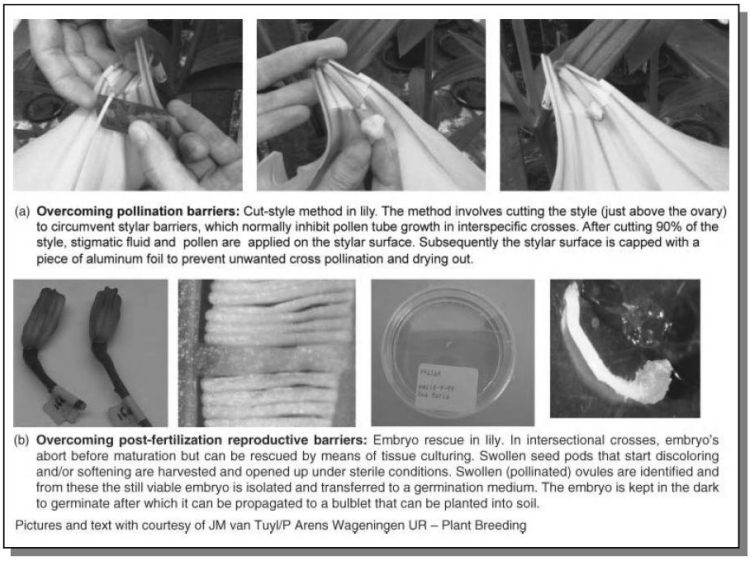
2) SPOROFITNA INKOMPATIBILNOST

Sporofitno inkompatibilnost prav tako določa lokus S z multipli aleli (S1, S2, S3, …). Vendar reakcijo peloda določa diploidni **genom somatskega tkiva starševske rastline peloda (sporofit).** Pri sporofitni inkompatibilnosti **S aleli kažejo določeno stopnjo dominance**, zato lahko nastane zigota, ki je homozigotna za S alel (bolj kompleksna kot gametofitna kompatibilnost). Značilna je za redkev, brokoli in listni ohrovt. Mesto inhibicije površina brazde.

Primer: rastlina z genotipom S1 in S2 (S1 dominanten). Nastali pelod deluje kot S1. Ta bo na S1 brazdi zavrnjen, vendar pa bo lahko kalil na S2 brazdi.

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Samo-inkompatibilnost lahko z določenimi postopki obidemo



(a) **Overcoming pollination barriers:** Cut-style method in lily. The method involves cutting the style (just above the ovary) to circumvent stylar barriers, which normally inhibit pollen tube growth in interspecific crosses. After cutting 90% of the style, stigmatic fluid and pollen are applied on the stylar surface. Subsequently the stylar surface is capped with a piece of aluminum foil to prevent unwanted cross pollination and drying out.

(b) **Overcoming post-fertilization reproductive barriers:** Embryo rescue in lily. In intersectional crosses, embryo's abort before maturation but can be rescued by means of tissue culturing. Swollen seed pods that start discoloring and/or softening are harvested and opened up under sterile conditions. Swollen (pollinated) ovules are identified and from these the still viable embryo is isolated and transferred to a germination medium. The embryo is kept in the dark to germinate after which it can be propagated to a bulblet that can be planted into soil.

Pictures and text with courtesy of JM van Tuyl/P Arens Wageningen UR – Plant Breeding

Figure 5.7 Overcoming reproductive barriers: (a) pollination barriers; (b) post-fertilization reproductive barriers.

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Uporaba sistemov inkompatibilnosti pri žlahtnjenju rastlin

Pridelava hibridnega semena!

a) ROČNI ALI MEHANSKI NADZOR OPRAŠEVANJA
(emaskulacija, mehansko odstranjevanje moških cvetov)

b) NADZOR S KEMIJSKO INDUCIRANO STERILNOSTJO

c) GENETSKI MEHANIZMI
Večina danes uporabljenih opraševalnih regulatornih mehanizmov za pridelavo hibridnega semena temelji na genetski regulaciji.

Poleg inkompatibilnosti še MOŠKA STERILNOST

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Chemicals which kill the male gametes, spores or organs and render the treated plant mst. These chemicals are known as male-gametocides or chemical hybridizing agents and the selective abolition of male sex is termed chemical male sterility (ch-mst).

Table 4.1. Gametocides used in inducing male sterility

No.	Name/abbreviation	Chemical name
1	6-BAP	6-Benzylaminopurine
2	CCC	Chlorocholine chloride (2-chloroethyl) trimethylammonium chloride
3	CCDP	3-Carboxy-1-(p-chlocophenyl) -4, 6, dimethyl-2 pyridine
4	Colchicine	An alkaloid
5	Coumarin	A bicyclic organic compound
6	Dalapon	Sodium 2, 2-dichloroporpionate
7	DPX	3-(p-Chlorophenyl)-6-methoxy-3-triazine-2, 4-(1H, 3H) dione
8	2, 4-D	2, 4-Dichlorophenoxy-acetic acid
9	DMA	Dimethylarsenic acid
10	Ethrel	2-Chloroethyl phosphonic acid
11	EB	Ethidium bromide
12	Estrone	2,7-Diamino-10-ethyl-9-phenylphenantridium bromide
13	Embutox-4	γ-(2, 4-Dichlorophenoxybutyric acid)
14	GA	Gibberellic acid
15	IAA	Indole-3-acetic acid
16	IBA	Indole butyric acid
17	MH	Meleic hydrazide (1, 2-dihydropyridazine-3, 6-dione)
18	Mendok	Sodium 2, 3-dichloro-2-methyl-propionate
19	Morphactin	Fluorine-9-carboxylic acid derivative
20	NAA	Naphthalene acetic acid
21	Phosphon	Tributyl-2, 4-dichlorobenzylphosphonium chloride
22	RH	Sodium p-(chlorophenyl)-1-2-dihydro-4, 6-dimethyl-2-oxonicotinate (and its derivatives)
23	SADH	Succinic acid, 2, 3 dimethyl hydrazide
24	Sodium nucleate	Sodium salt of nucleic acid
25	TIBA	2, 3, 5-Tri-iodobenzoic acid
26	ZMA	Zinc methyl arsenate

Table 4.2. Chemical male sterility induction in some plants

Species	Chemical number[a]
Abelmoschus esculentus	17, 18, 14
Allium cepa	6, 14, 17, 21, 24
Antirrhinum majus	18
Avena sativa	7, 14, 17
Beta patula	18
B.vulgaris	7, 9, 10, 12, 18
B.webbiana	18
Brassica napus	26
B.oleracea	14
Cajanus cajan	17, 18
Cannabis sativa	1, 10
[a]Callistephus sinensis	17

Kaul. 1988. Chemical Male Sterility. Male sterility in higher plants.

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Pomen inkompatibilnosti pri kulturnih rastlinah

Pridelava hibridnega semena, vendar je lahko težavna pridelava inbridiranih linij (starševskih linij). Primer: zelje

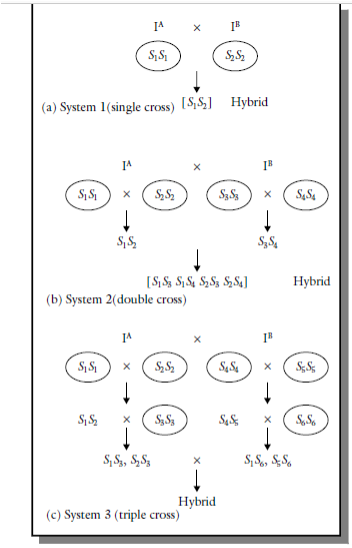


Figure 5.8 Application of self-incompatibility in practical plant breeding. Sporophytic incompatibility is widely used in breeding of cabbage and other Brassica species. The single cross hybrids are more uniforms and easier to produce. The top cross is commonly used. A single self-incompatible parent is used as female and is open-pollinated by a desirable cultivar as pollen source.

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MOŠKA STERILNOST (posledica nefunkcionalnosti prašnikov ali peloda)

- PRAVA MOŠKA STERILNOST (enospolni cvetovi, dvospolni cvetovi z nefunkcionalnimi mikrosporami)

Glede na izvor ločimo naslednje oblike sterilnosti:

- GENETSKA MOŠKA STERILNOST (tudi jedrna ali genska)
- CITOPLAZEMSKA MOŠKA STERILNOST
- CITOPLAZEMSKA GENETSKA MOŠKA STERILNOST
- FUNKCIONALNA MOŠKA STERILNOST (pelod je fertilen vendar zaradi nepravilnega razvoja prašnic ni sproščen)

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GENETSKA (JEDRNA) MOŠKA STERILNOST (GMS)

- gen za sterilnost najden pri več vrstah npr. ječmen, bombaž, soja, paradižnik, krompir, limski fižol.
- ne pride do razvoja peloda ali prašnikov.
- običajno pogojena z **recesivnim jedrnim genom „ms“, medtem ko dominantni „Ms“ kodira normalen razvoj prašnic in peloda.**
- nemogoče pridobiti populacije s 100 % moško sterilnostjo, zato je potrebno fertilne rastline pred cvetenjem odstraniti (morfološke lastnosti vezane na sterilni lokus ali uporaba molekulskih markerjev)

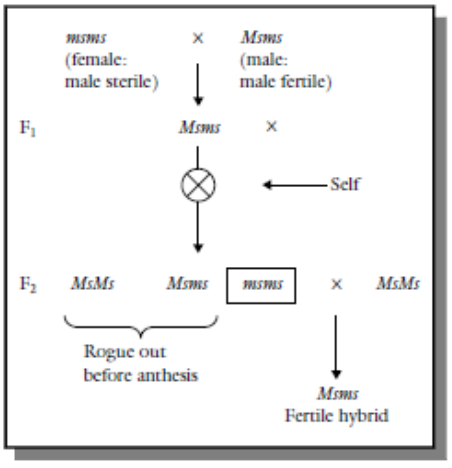


Figure 5.9 Genetic male sterility as used in practical breeding.

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CITOPLAZEMSKA MOŠKA STERILNOST (CMS)

- Kontrolirana z mitohondrijiskimi geni
- Dedovanje citoplazme – po materni strani
- možen prenos CMS s tehnikami tkivnih kultur: prenos jedrnih kromosomov v tujo citoplazmo
- Najdena pri koruzi, sirku, sladkorni pesi, korenju, lanu.
- Pomemben tudi pri žlahtnjenju okrasnih rastlin (rastline, ki ne razvijejo plodov cvetijo dlje časa)

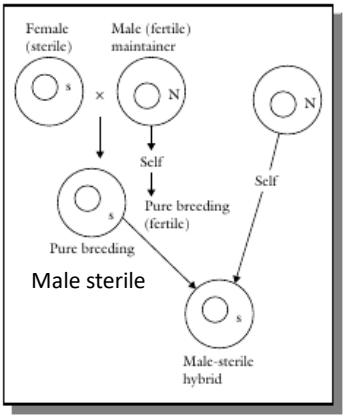


Figure 5.10 Cytoplasmic male sterility as applied in plant breeding (N, normal cytoplasm; s, sterile cytoplasm).

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CITOPLAZEMSKO-GENETSKA MOŠKA STERILNOST

- Poleg citoplazemskega zapisa, imajo pri tej sterilnosti pomen tudi restorerski geni v jedru.
- **V kolikor je v jedru prisoten „Rf" restorerski gen, je omogočena normalna sinteza peloda.**
- Uporabljena za pridelavo hibridnega semena sirka, sončnic, koruze, riža, sladkorne pese, itd.

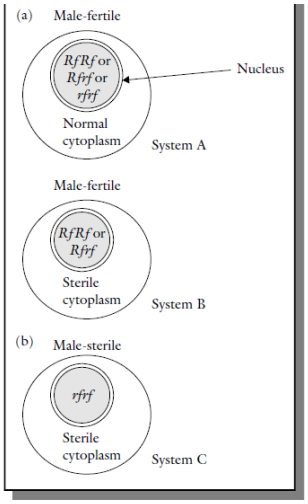


Figure 5.11 The three systems of cytoplasmic genetic male sterility. The three factors involved in CMS are the normal cytoplasm (N), the male sterile cytoplasm (S), and the fertility restorer (*Rf*, *rf*).

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Običajno moramo pri pridelavi hibridnega semena imeti naslednje linije:

A-linija: CMS linija – sterilna citoplazma in z nerestorerskimi geni (S(rf rf)) (materin starš)
(linija za pridobivanje hibridnega semena)

B-linija: ohranjevalna – N citoplazma – (rf rf)
(za ohranjanje A linije)

R-linija: restorerska linija –nosi gene (N(Rf Rf), S(RfRf)) – očetovski starš

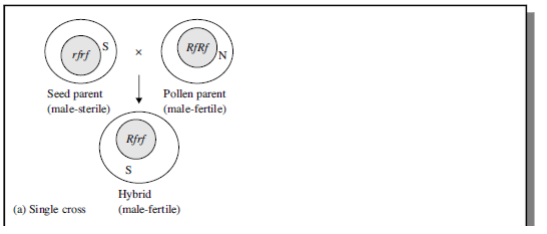


Figure 18.1 Breeding by CMS. (a) single cross

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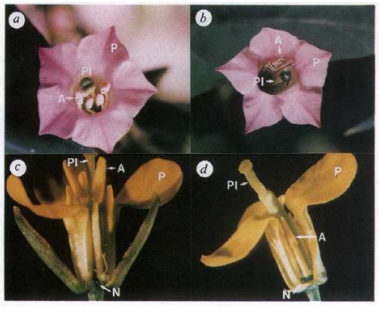
21

Transgena moška sterilnost

Induction of male sterility in plants by a chimaeric ribonuclease gene
Nature **347**, 737-741 (25 October 1990)

Chimaeric ribonuclease genes that are expressed in the anthers of transformed tobacco and oilseed rape plants were constructed. Chimaeric ribonuclease gene expression within the anther selectively destroys the tapetal cell layer that surrounds the pollen sac, prevents pollen formation, and leads to male sterility. These nuclear male sterility genes should facilitate the production of hybrid seed in various crop plants.

FIG. 3 Male sterile tobacco and oilseed rape flowers. *a* and *b*, Tobacco flowers from untransformed plants (*a*), and plants transformed with *TA29-RNase* gene (*b*). A, P and Pl, anther, petal and pistil, respectively. *c* and *d*, Oilseed rape flowers from untransformed plants (*c*), and plants transformed with a *TA29-RNase* gene (*d*). A, P, Pl and N, anther, petal, pistil and nectar, respectively.
METHODS. The *TA29-RNase T1 and TA29-barnase* genes were transferred to a binary vector[26] containing the *bar* gene[18]. Oilseed rape hypocotyls (*Brassica napus* cv. 'Drakkar') were transformed with *Agrobacterium* according to the procedure of De Block *et al.*[27] using bialaphos resistance (*bar*) as a selectable marker.



ribonukleaza (Barnase) in restorer – inhibitor ribonukleaze (Barstar)

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