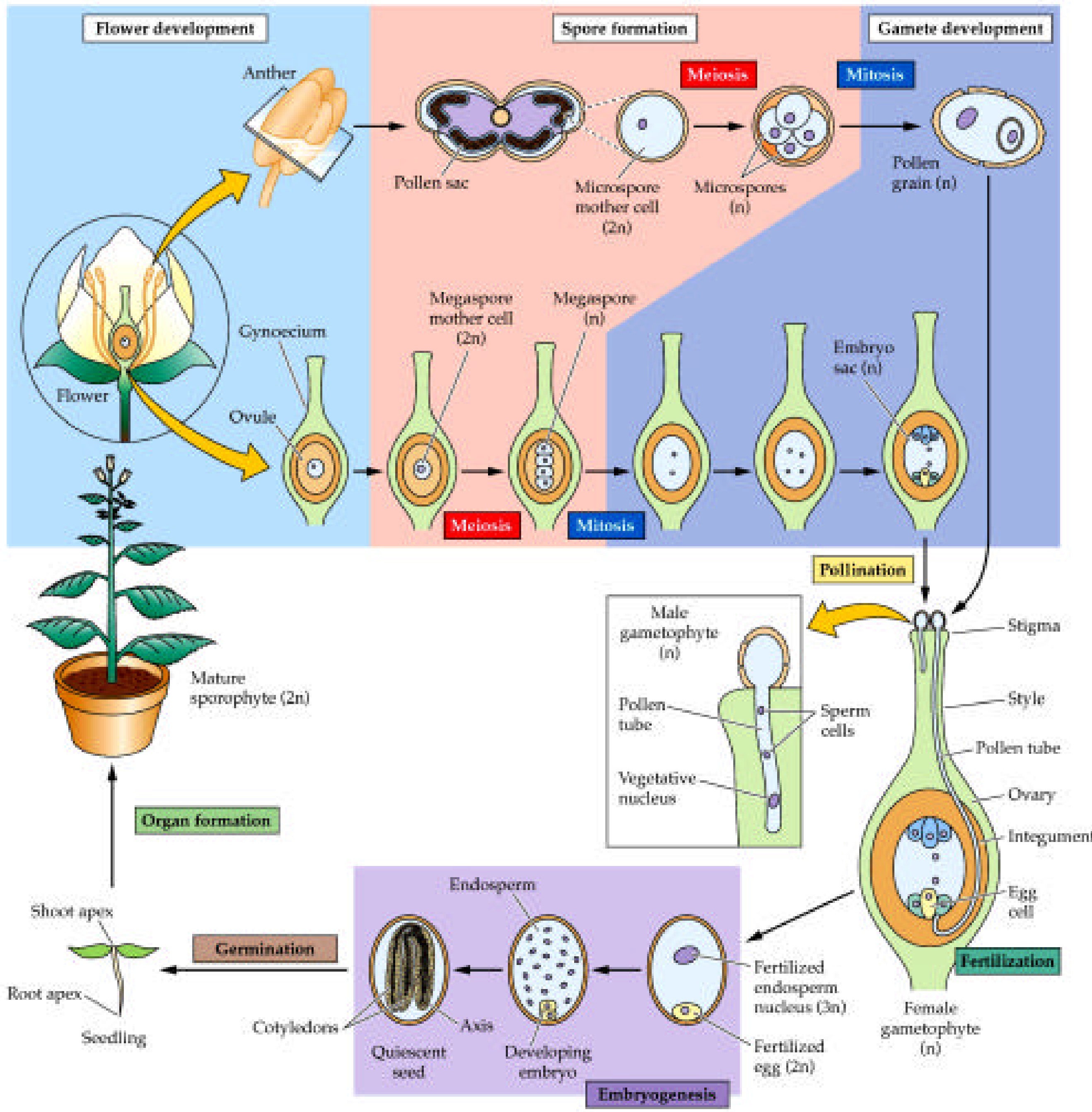
Lecture 22

Gametogenesis, fertilization, seed formation and biotechnological applications

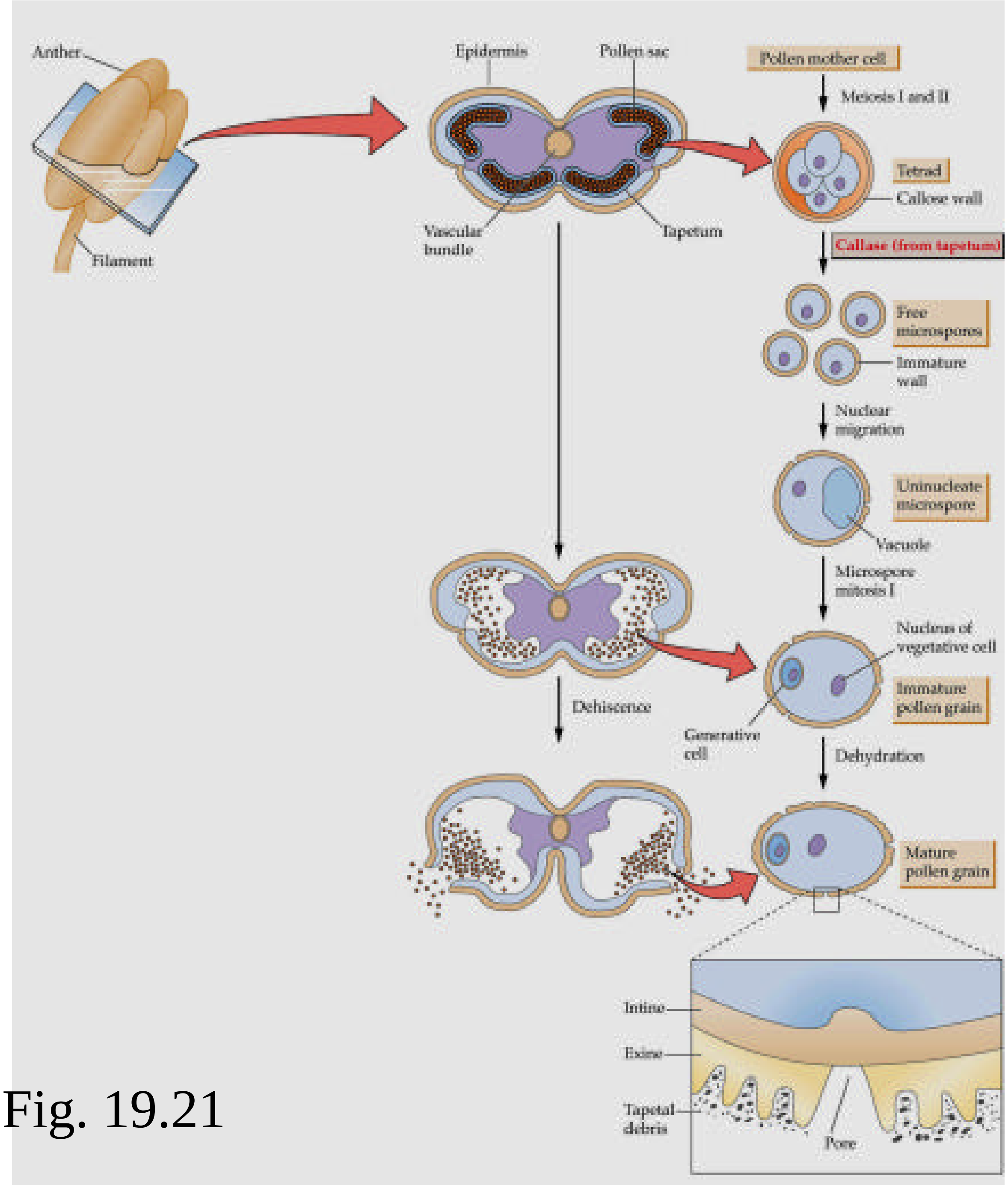
Read 1006-1018, 1022-1032

Sporophytic
Gametophytic

Fig. 19.1



Flower development
Spore formation
Gamete development
Anther
Meiosis
Mitosis
Pollen sac
Microspore mother cell (2n)
Microspores (n)
Pollen grain (n)
Flower
Gynoecium
Ovule
Megaspore mother cell (2n)
Megaspore (n)
Embryo sac (n)
Meiosis
Mitosis
Pollination
Male gametophyte (n)
Pollen tube
Sperm cells
Vegetative nucleus
Stigma
Style
Pollen tube
Ovary
Integument
Egg cell
Fertilization
Female gametophyte (n)
Mature sporophyte (2n)
Organ formation
Endosperm
Fertilized endosperm nucleus (3n)
Fertilized egg (2n)
Axis
Cotyledons
Quiescent seed
Developing embryo
Embryogenesis
Germination
Shoot apex
Root apex
Seedling



Anther
Filament
Epidermis
Pollen sac
Vascular bundle
Tapetum
Pollen mother cell
Meiosis I and II
Tetrad
Callose wall
Callase (from tapetum)
Free microspores
Immature wall
Nuclear migration
Uninucleate microspore
Vacuole
Microspore mitosis I
Nucleus of vegetative cell
Immature pollen grain
Generative cell
Dehiscence
Dehydration
Mature pollen grain
Intine
Exine
Tapetal debris
Pore

Fig. 19.21

Female gametophyte development

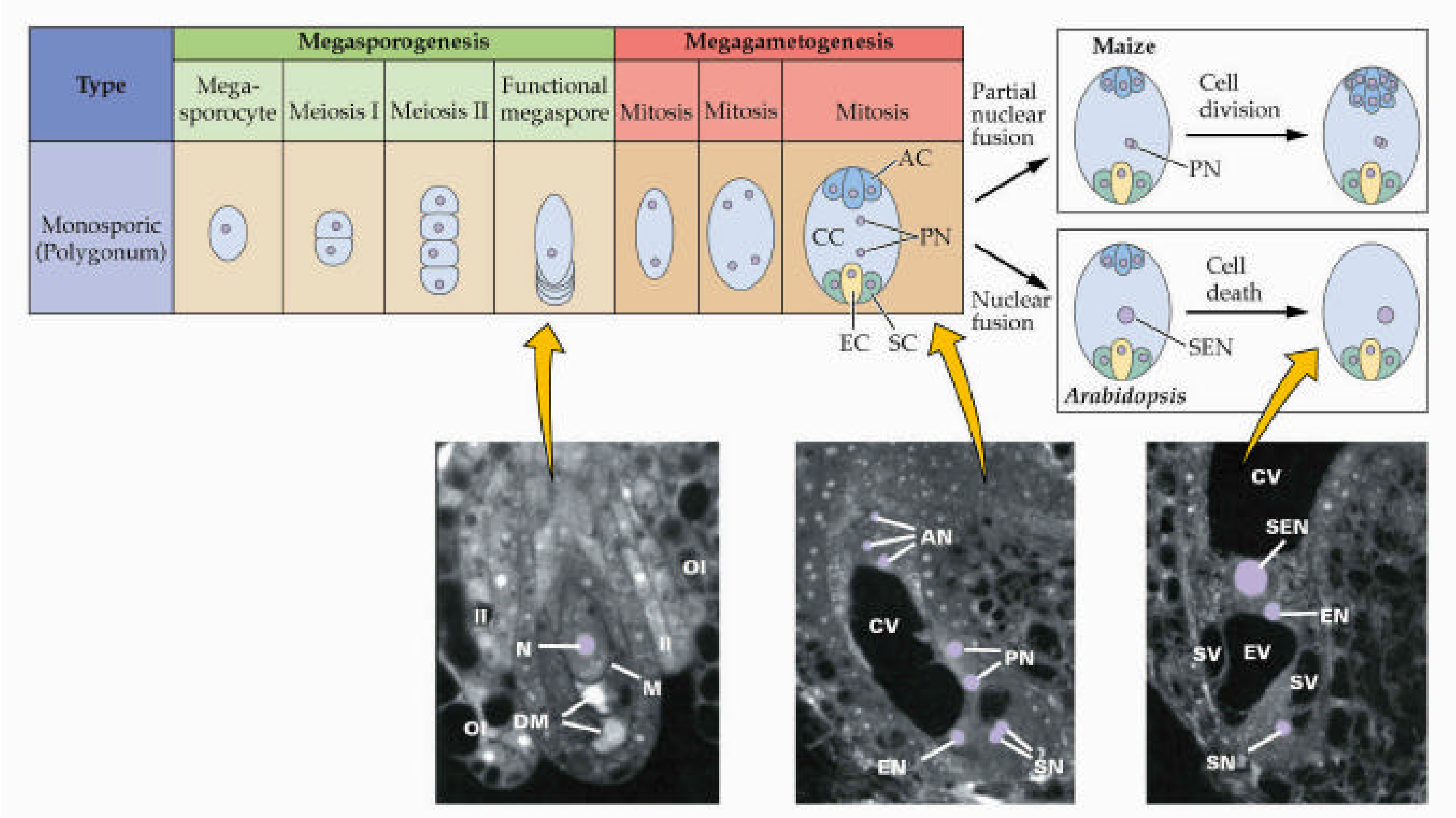


Fig. 19.24

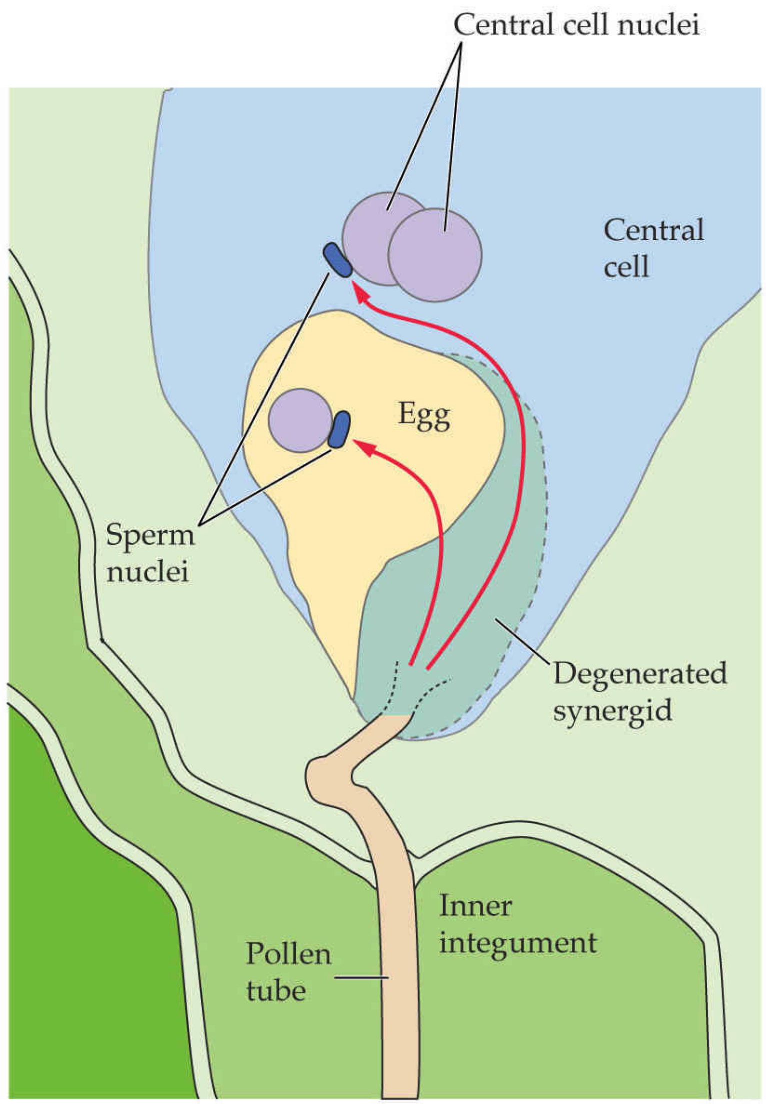


Fig. 19.35 Doublr fertilization

EP: Embryo proper
S; Suspensor
Pd: Proctoderm--Ed: epidermal
Gm: Ground Meristem--storage parenchyma (P)
Pc: procambium --Vascular tissue (V)
Hs: hypophysis--root and shoot meristem

A:axis
C: cotyledons

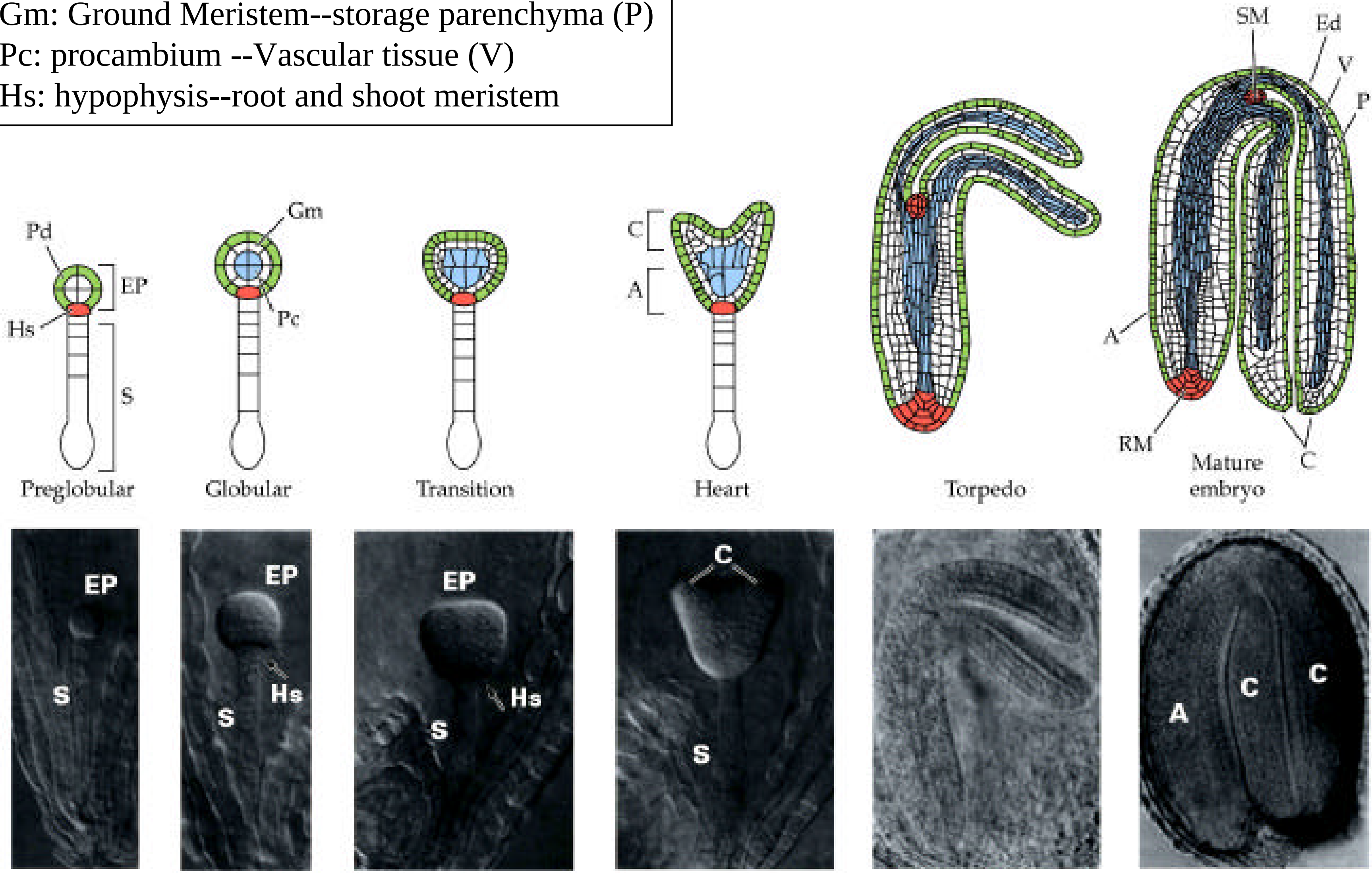
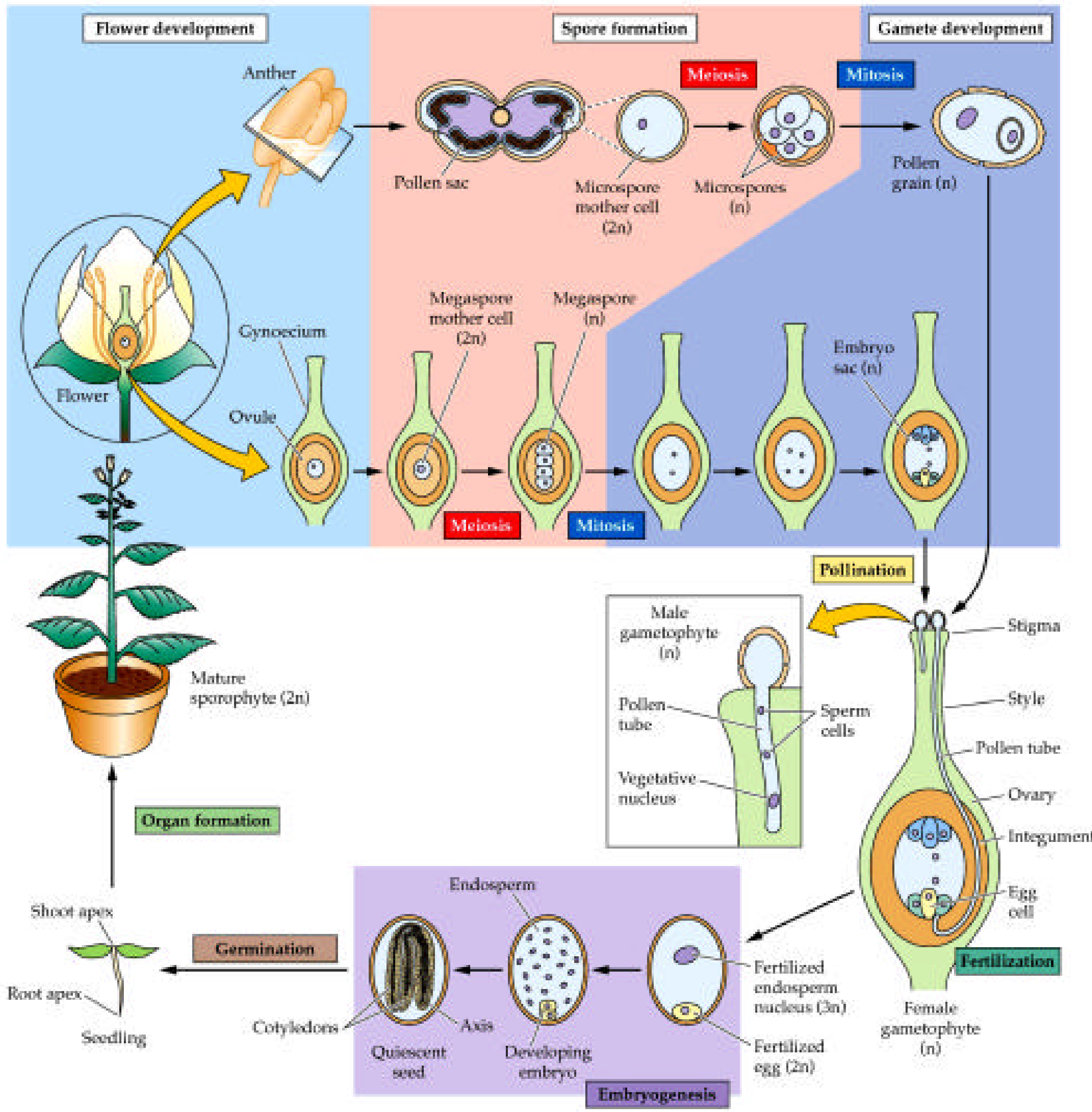


Fig. 19.36

Fig. 19.1



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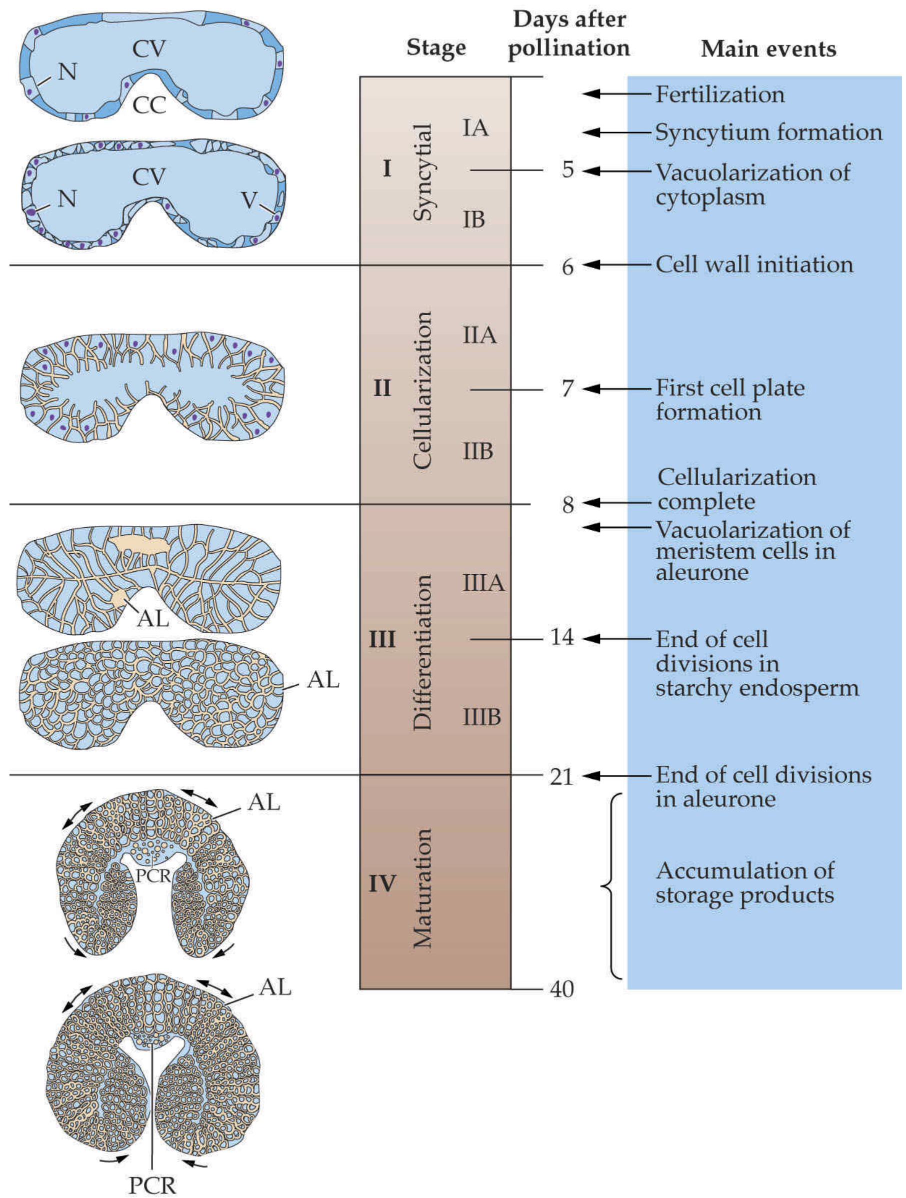


Fig. 19.38 endosperm development

Storage reserves of some important crop species

Average percent composition

	protein	oil	carbohydrate	major storage organ
Cereals				
Barley	12	3	76	Endosperm
Maize	10	5	80	Endosperm
Oats	13	8	66	Endosperm
Wheat	12	2	75	Endosperm
Legume				
Broad bean	23	1	56	Cotyledons
Garden pea	25	6	52	Cotyledons
Peanut	31	48	12	Cotyledons
Soybean	37	22	12	Cotyledons
Other				
castor bean	18	64	negligible	Endosperm
oil palm	9	49	28	Endosperm
pine	35	48	6	Megagametophyte
Papeseed	21	48	19	Cotyledons

Seed storage protein- major proteins in seed

1. multiple forms within one species

2. only produced in seed

3. stored in protein bodies

4. types:
   - -albumins: water soluble
   - -prolamins: alchohol/water soluble
   - -globins:weak saline solution soluble

Examples:

1. albumins -mostly in dicots

2. Prolamins
   - -primarily in grasses
   - - internal repeats of amino acids, glutamine and proline rich
   - -S-rich- repeats in N-term
   - -S-poor- few cysteins
   - -High MW

3. Globins
   - -in both monocots and dicots
   - -low in methionine and cysteine
   - -e.g legumins in legumes

Structure of seed storage proteins

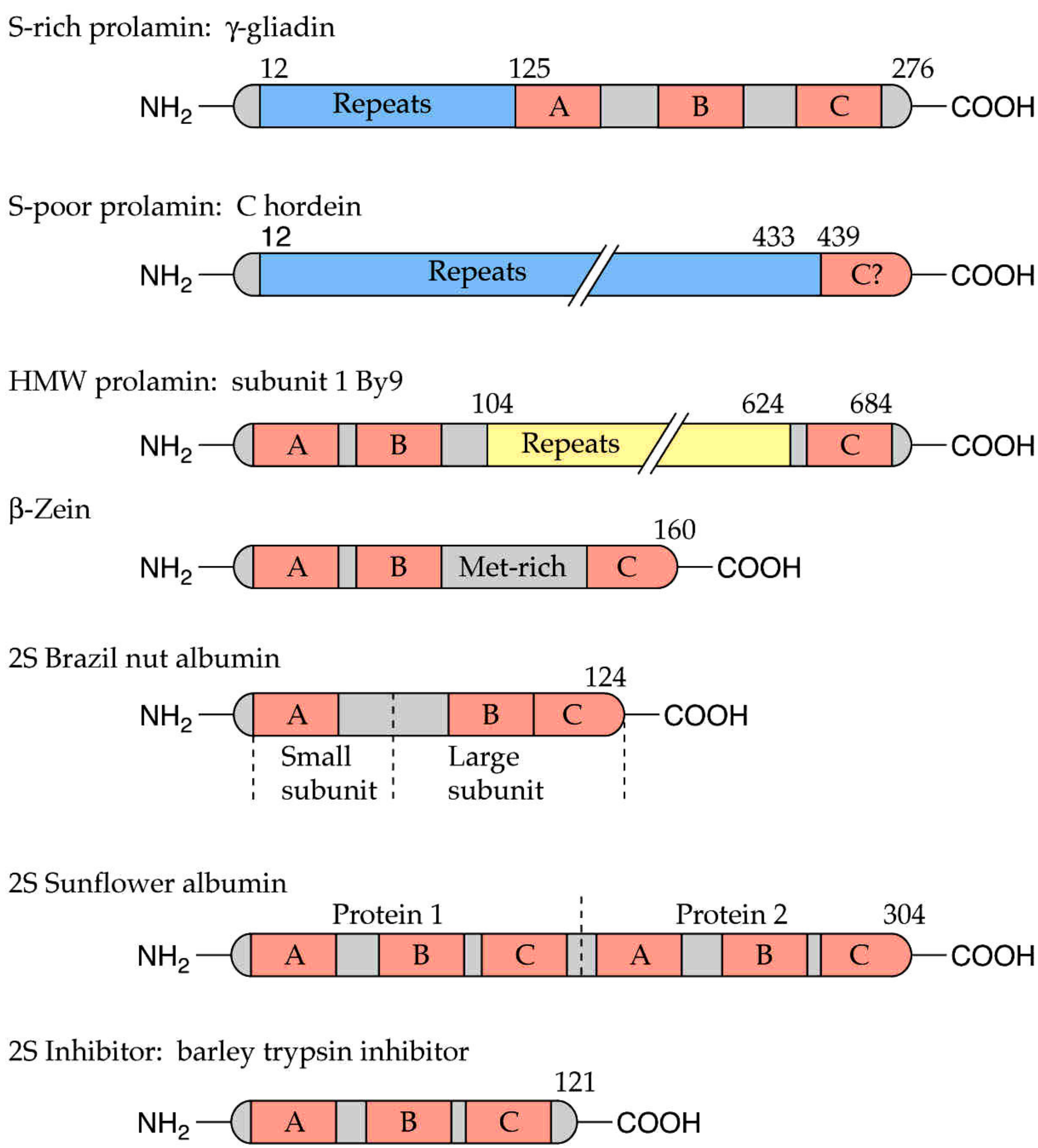
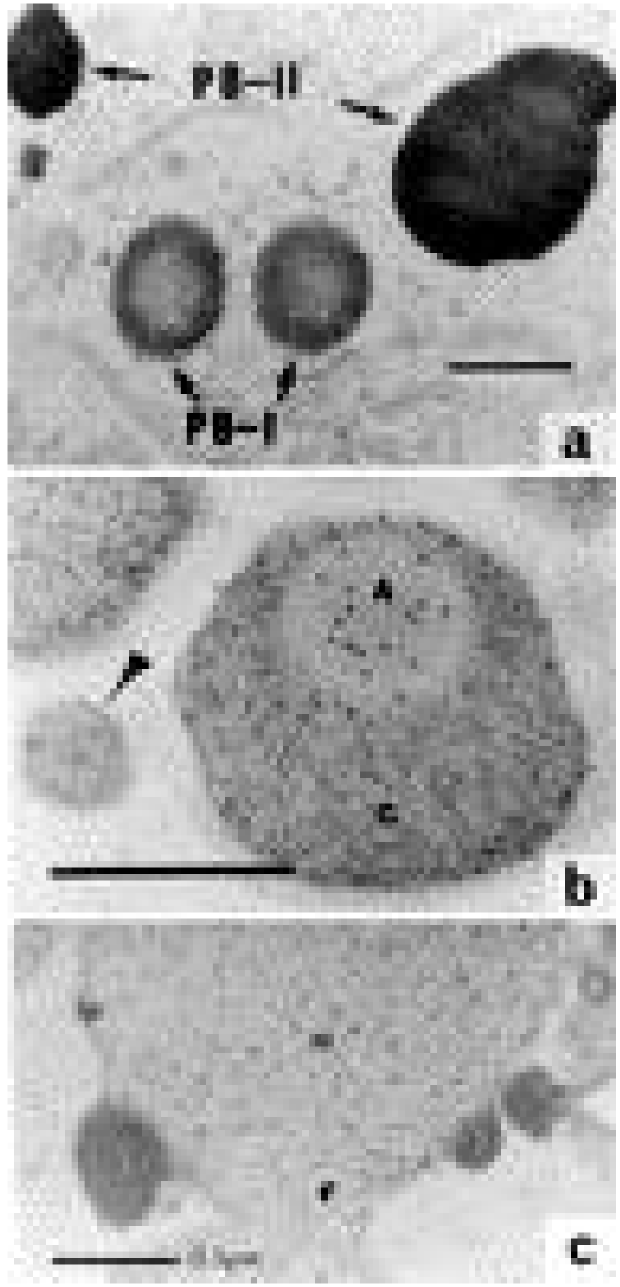


Fig. 19.41

Rice

Oats

wheat



Engineering Plants to Improve Food Quality

I. Engineering improved nutrition in seed-storage proteins

A. problem- unbalanced essential amino acids in seeds

-legumes- low in S-containing aa- cysteine and methionine
-cereals- low in lysine, threonine, tryptophane

B. Modifications to improve quality

1. Modify sequence of native storage protein

2. Express another plant's seed storage protein
   - -e.g. a. Brazil nut 2S albuminexpressed in tobacco and rape (33% more methionine)
   - b. Phaseolin (Bean) expressed in rice

3. Changing balance of native genes
   - -antisense of one form that is low in nutrition may enhance other form
     - -e.g. Rape seed
       - -cruciferin-12S albumin- 60% of protein
         albumin- low in methionine and cysteine
       - -napin- 2S albumin- higher met and cyst
     - -antisense of cruciferin gene- reduced cruciferin, increased napin

II. Engineering gluten in wheat

-gluten- a prolamin (1/2 of total protein) found in wheat
- -allows elasticity-needed for bread making
- -50 genes producing glutens

-two types of prolamins-
- gliadins-provide viscosity
- glutenins (HMW prolamins)- -provide elasticity

-forms continuous matrix (network) in endosperm cells, conferring visco-elastic properties

-high molecular mass glutenin polymers ;eads to strong doughs

-Genetics- in hexaploid bread wheat-has six HMW subunit genes
- 2 genes (x and y) at each of 1A, 1B, 1D on chromosome one
- 1Ax subunit is associated with good bread

-characterized from cloning of good and poor quality
- -expression of protein
- -more cysteins in better quality alleles(S-s bond for network)

-Improvements:
- -introduce high quality alleles by expressing 1Ax1 transgene
- -fine tune quality

Golden Rice

It is estimated 124 million children worldwide lack vitamin A, putting them at risk of permanent blindness and other serious ailments.

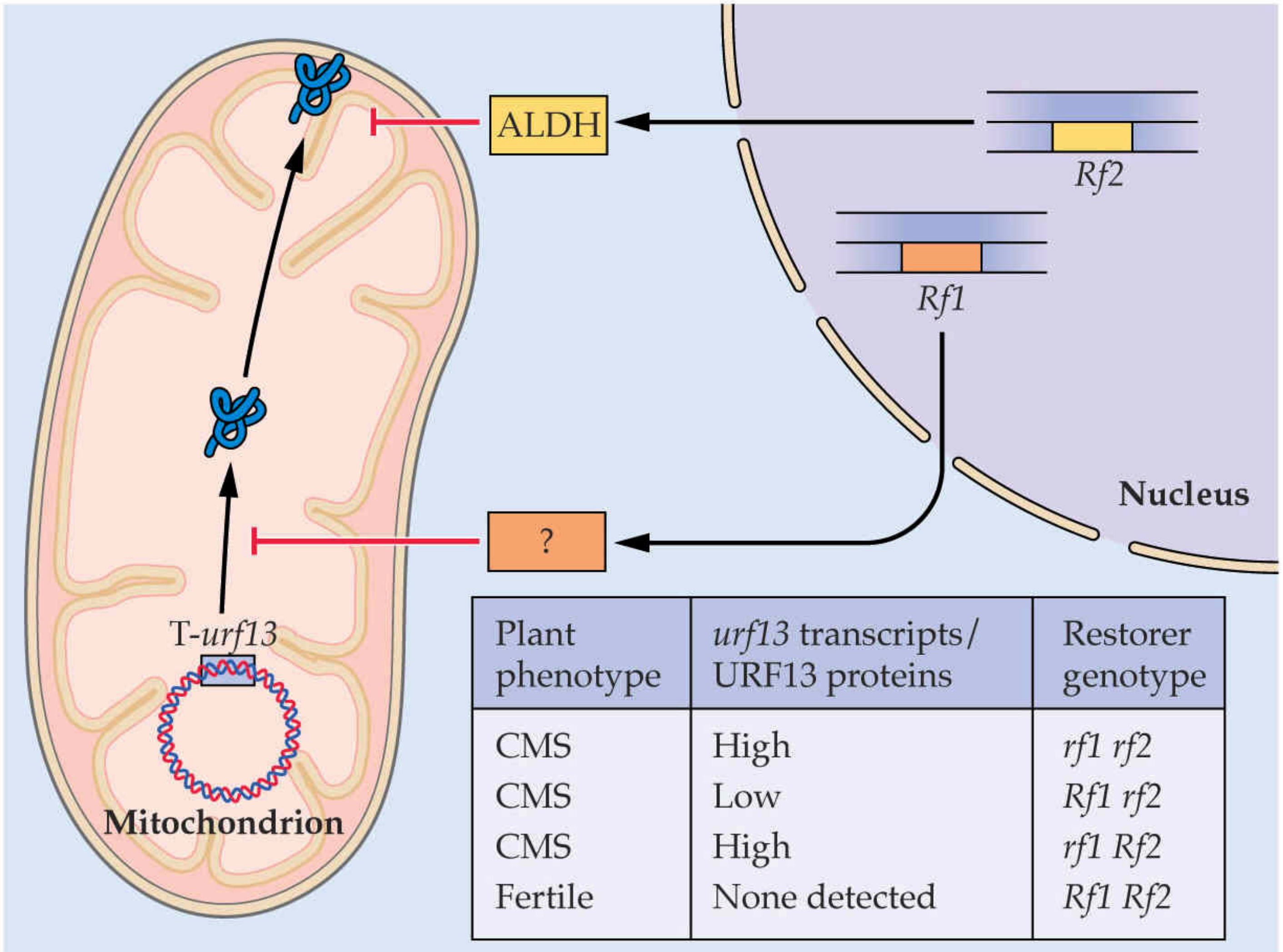
The scientists (Ingo Potrykus), based at the Swiss Federal Institute of Technology in Zurich, inserted three genes into rice that make the plant produce beta-carotene.

Geranylgeranyl diphosphate — **Phyteone synthase** — Phytoene desaturase / ζ -carotene desaturase / Lycopene β-cyclase → **B-carotene (provitamin A)**

Gt1: glutelin promoter (endosperm specific) and 35S promoter

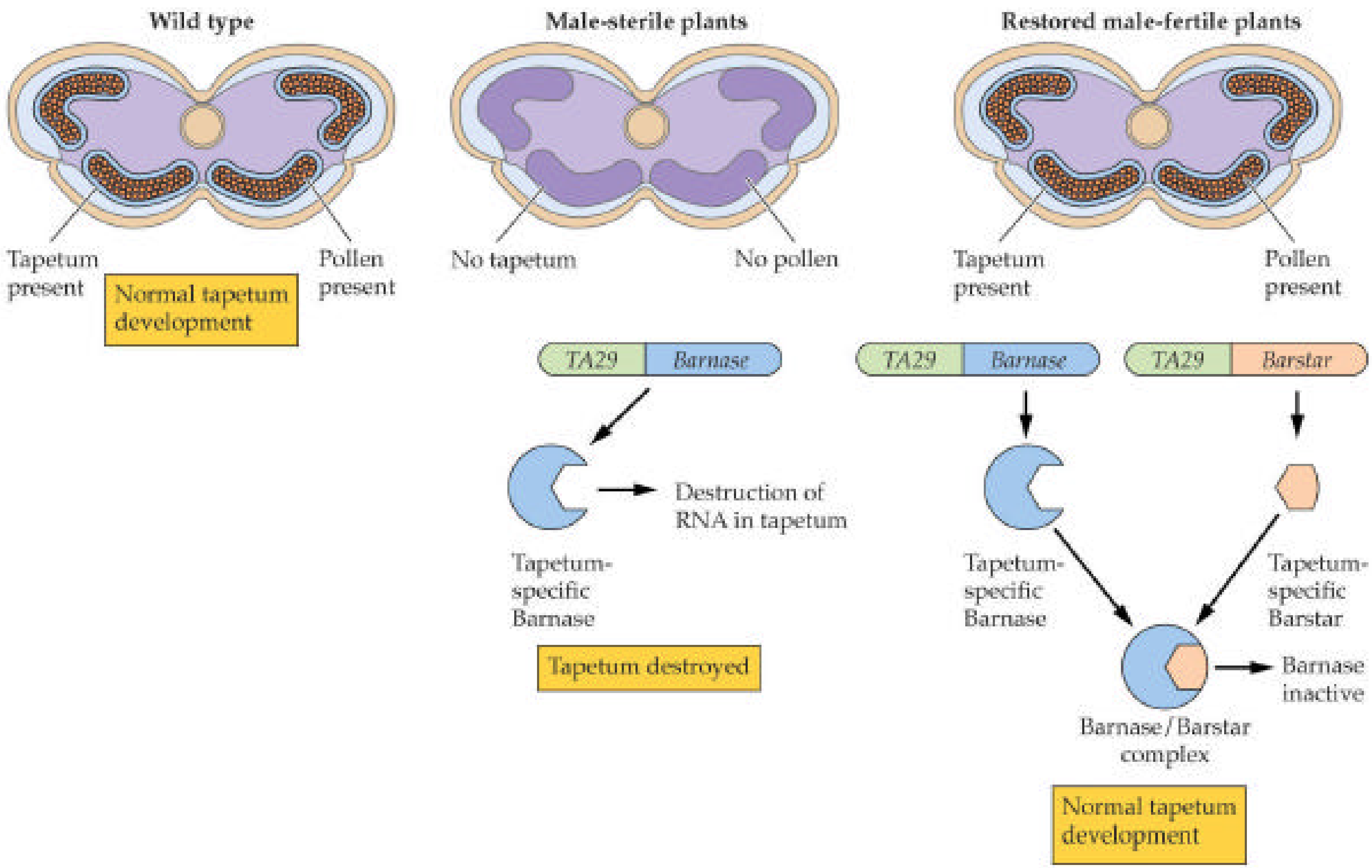
http://www.csa.com/hottopics/gmfood/webpage10.html

Cytoplasmic male sterility (CMS) and hybrid production



Plant phenotype	*urf13* transcripts/ URF13 proteins	Restorer genotype
CMS	High	*rf1 rf2*
CMS	Low	*Rf1 rf2*
CMS	High	*rf1 Rf2*
Fertile	None detected	*Rf1 Rf2*

Hybrid seed production using biotechnology



Box. 19.5

Lecture 23: Edible vaccines, designer food and ethical issues

http://www.sciam.com/2000/0900issue/0900langridge.html

Modification for food processing and taste

Delayed ripening of tomato

Flavr Savr™ tomato--Calgene

Polygalacturonase (PG) belonging to the petinase enzyme family

PG breaks down pectin--major cell wall component--solid to soft tissue

Antisense to PG--softening process stops

--other ripening processes (flavor and color) not affected

--firmer tomato with reduced water content are slow to rot

--more concentrated when processed to paste (Campell's soup)

--reduced damage in transit, fewer fungicide

--contain kanamycin resistance

--on market 1994 _

Zeneca's tomato using gene sense suppression to PG

mostly used in tomato puree

<u>Up to 50% fruits & vegetables are lost to spoilage</u>

Ethylene controls the ripening of climacteric fruits

- i.e. fruits that changes their pattern of respiratory gases during ripening
- such as banana, tomato, apple, pear, mango and melon
- non-climacteric fruits: oranges, lemon, strawberry

Inhibiting ethylene synthesis could inhibit ripening in climacteric fruits

Target genes: ACC synthase & ACC oxidase

Improvement in quality, storage life, appearance & nutritional value of clim. fruits

Healthier French fries

A starch-producing gene from E. coli ADP-glucose pyrophosphorylase
was inserted into potato genome-- 20% more starches, less water
In fries, this potatoes absorb less oil (30% vs 36%), healthier, quick frying time

Increase sweetness

Thaumatin, a protein found in katemfe plant in Africa--put into potatoes
Monellin , from serendipity berries--put into tomato and lettuce
Calgene has gene that was put into strawberries
Both proteins are 3000X the sweetness of sucrose
Designer taste in agriculture products for tomorrow's supermarket

Pipless fruits

SDLS-2, found in many plants, kill unwanted cells during plant development
This gene is fused to seed-specific promoters to destroy seeds--pipless oranges

Blue jeans

Cotton is one of the most successful GM crops
(1/4 US cottons are from transgenic seeds, resistant to insects or herbicides)
Clothes made from these cottons are exported arround the world
Cotton plants that contain foreign genes expressing blue pigment--blue jeans
Colored lint from cotton plants already obtained, reduce the need for dying

Ecological risks

(Much studies are needed in this area)

Spread to the wider environment-impossible to eradicate

-Gaining vigor or invasiveness, become weeds of agricultural or natural habitat such as exotic introductions-achieve population growth impossible in native habitat

-Transferred to wild relative, hybrid offspring detrimental to existing flora or fauna

Seeds from Herbicide resistance plant could remain in the soil
grown the following year, within subsequent crops, in the same field
They are difficult to kill because of herbicide resistance
Or they may threaten wild population of related plants by competition

Pollen of oilseed rape (*Brassica napus*) can fertilize plants up to 2.5 km away
And can fertilize its wild relative *Brassica campestris*
The hybrid is weed-like and high fertility

Bt-resistant insects

Risk to human health

-Allergens--food allergies--consuming substances that trigger reaction in digestive system: vomiting or diarrhoea; whole body: eczema and urticaria

Most common: milk, peanuts, eggs, shellfish, soya, cereals ect.
Genetic engineering can be used to remove the allergic protein from food
Such as a Japanese group was able to remove an allergic protein from rice.

FDA rule: GM food must be tested for allergy sensitivity if the genes are from food known to cause allergic reactions such as Brazil nuts.

-Antibiotic resistant microorganisms

.transfer the antibiotic marker gene into gut microflora in human or animals thus making certain clinical or veterinary antibiotics less effective

.higher risk in food containing live bacteria, less risk for plant materials least likely for highly processed foods.

.antibiotic resistant microorganism can be transferred from animals to human this is of concern if transgenic crops were widely used in livestock feed

Moral & ethical issues

-Transfer of human genes to animals used as food

-Transfer of certain animal genes whose flesh is forbidden as food

-Animal genes transferred to plants--still vegetarian food?

.Vegetarian cheese is produced using yeast containing a chymosin gene, alternative to cheese made from calf rennet

.An antifreeze gene was introduced from flounder into tomato to prevent frost damage

Can you patent life? Is DNA life?

Mandatory labeling

<u>Food industry is against it:</u>

final food products are no different
tranditional methods of genetic change does not require
food label in general pertains to composition not manufacturing process
unjustly stigmatize GM food
require segregation of GM crop--increase the cost, new distribution system
may jeopardize continued development of GM food
barrier to trade

<u>Consumer’s right to choose:</u>

see fundamental difference in gm food
unpredictable changes in food composition
right to choose
ethical or moral objections to genetic engineering
unexpected risk
contain antibiotic resistant gene
boycott McDonalds french fries, Quaker oats corn meal, CocaCola, Similac

Novel food and food ingredient regulation-EU

Required labeling if:

- Contain viable GMO
- Ingredients differ from existing ingredients
- contain materials that were not present in the original foodstuffs
- animal genes-ethical concerns

1997, nov: EC-mandatory labeling come into effect

Negative labeling: GMO-free