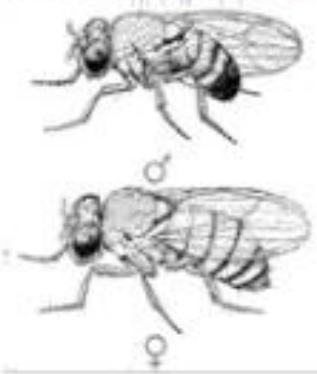
Mutation Breeding

Historical Account

- *Hugo de Vries: 1900. introduced the term "Mutation".*
- *Mutants were observed before this: e.g., short legged sheep was discovered by an English Farmer in 18th century. This sheep was used to develop a breed-c/a Ancon.*
- *Muller-discovered the action of X-rays on Drosophilla and Stadler discovered the effect of gamma-rays in barley.*
- *Mutation Breeding Programme was started in Sweeden, USSR and Germany in 1927 after the discovery by Muller.*



Mutation - Mutant

- Mutation

  Changes in genes and chromosomes
- Mutated

  Altered genes
- Mutant

  New organism with a mutated gene or rearranged chromosomes





Mutation breeding is successful when……

- Desired variability exhausts in cultivated species and germplasm.
- When a desirable variety has an oligogenic genetic defect.
- There is tight linkage between desirable and undesirable traits.
- Only one or two characters are to be improved in a fruit crop without changing its taste.
- Crop does not have sexuality, thus lacks variability.
- The generation cycle is very long, such as plantation crops, fruit trees….there mutation breeding is the shortcut way for genetic improvement.
- In ornamental plants


Mutant Dog Rose Flower


Mutant yellow Rose


Red Rose

Application of mutation breeding

- Induction of desirable mutant alleles which may not be present in the germplasm available to the breeder.
- In improving specific characteristics of well adapted high yielding variety .
- Mutagenesis has been successfully used to improve various quantitative characters including yield.

* **Can apply selection to single cells**

Limitations

- The frequency of desirable mutants is very low.
- Desirable mutations are commonly associated with undesirable side effects.
- There may be problems in the registrations of a mutant variety.
- Mutations in quantitative traits are usually in the direction away from the selection history of the parent variety.
- Most of the mutations are recessive.

* **Many mutations are non-heritable**

Effects Of Mutation

- Lethal :- They kill each & every individual that carry them in appropriate genotype .
  - Dominant lethal : It can't survive.
  - Recessive lethal : kill in homozygous state.

Sub Lethal & Sub Vital :- Both mutation reduce viability but don't kill all the individual carrying them in appropriate genotype.

- Sub Lethal : Kill more than 50%.
- Sub Vital : Kill less than 50%.

Vital :-
a) Don't reduce the viability.
b) Crop improvement can utilize only such mutations.

Lethal , Sub lethal mutations have no value in crop improvement.

Genetic structure changes

- Gene (point mutation)
- Chromosome
- Genome

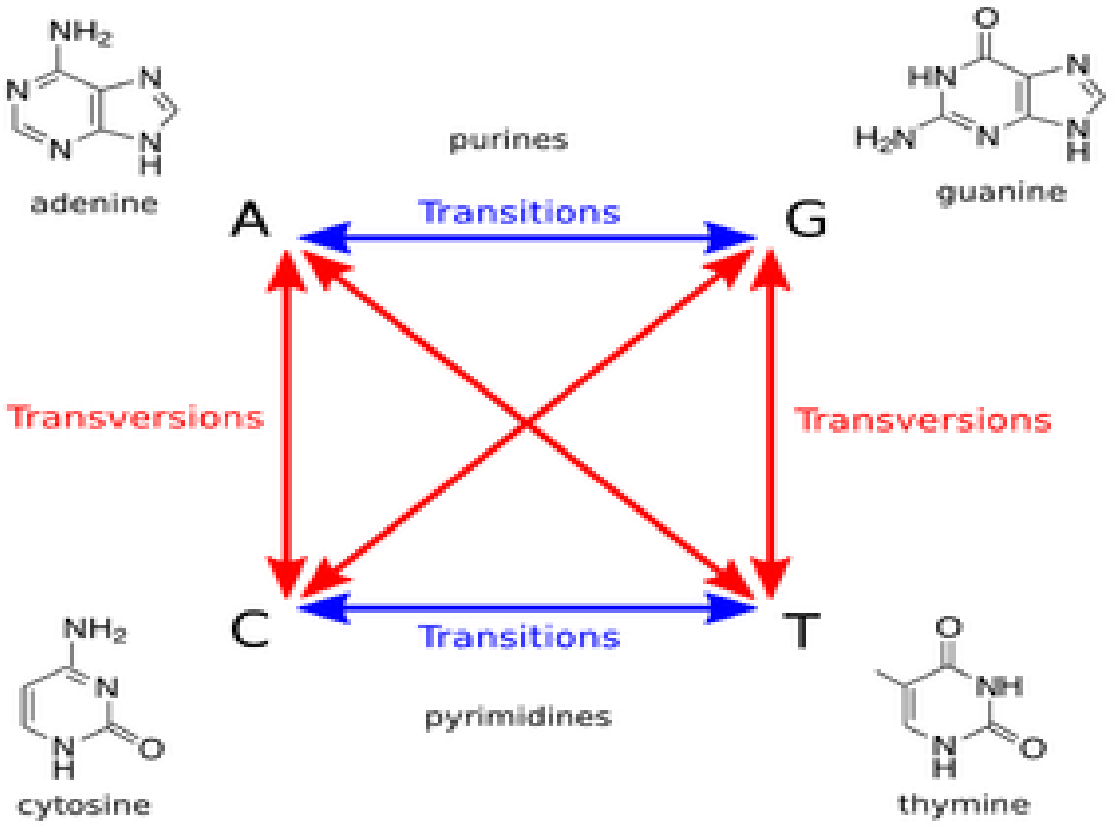
Gene (point) mutation

→ a change in specific sequence of nucleotides in DNA molecules leading to the formation of a new type of protein or preventing that of the normally protein

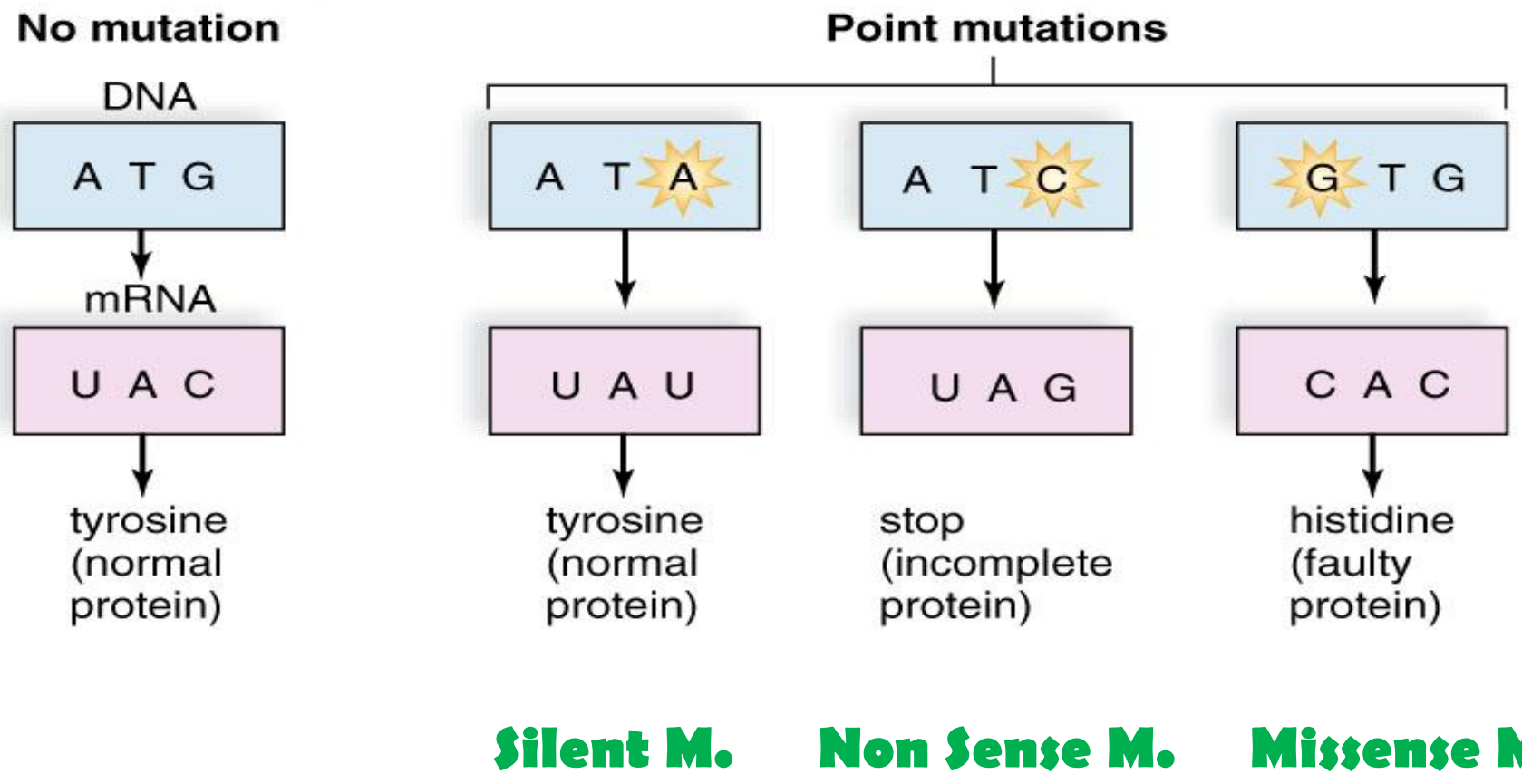
→ take place at the molecular or sub-microscopic level

→ Such change may be accompanied by the emergence of a new trait inherited in accordance with Mendel's Laws

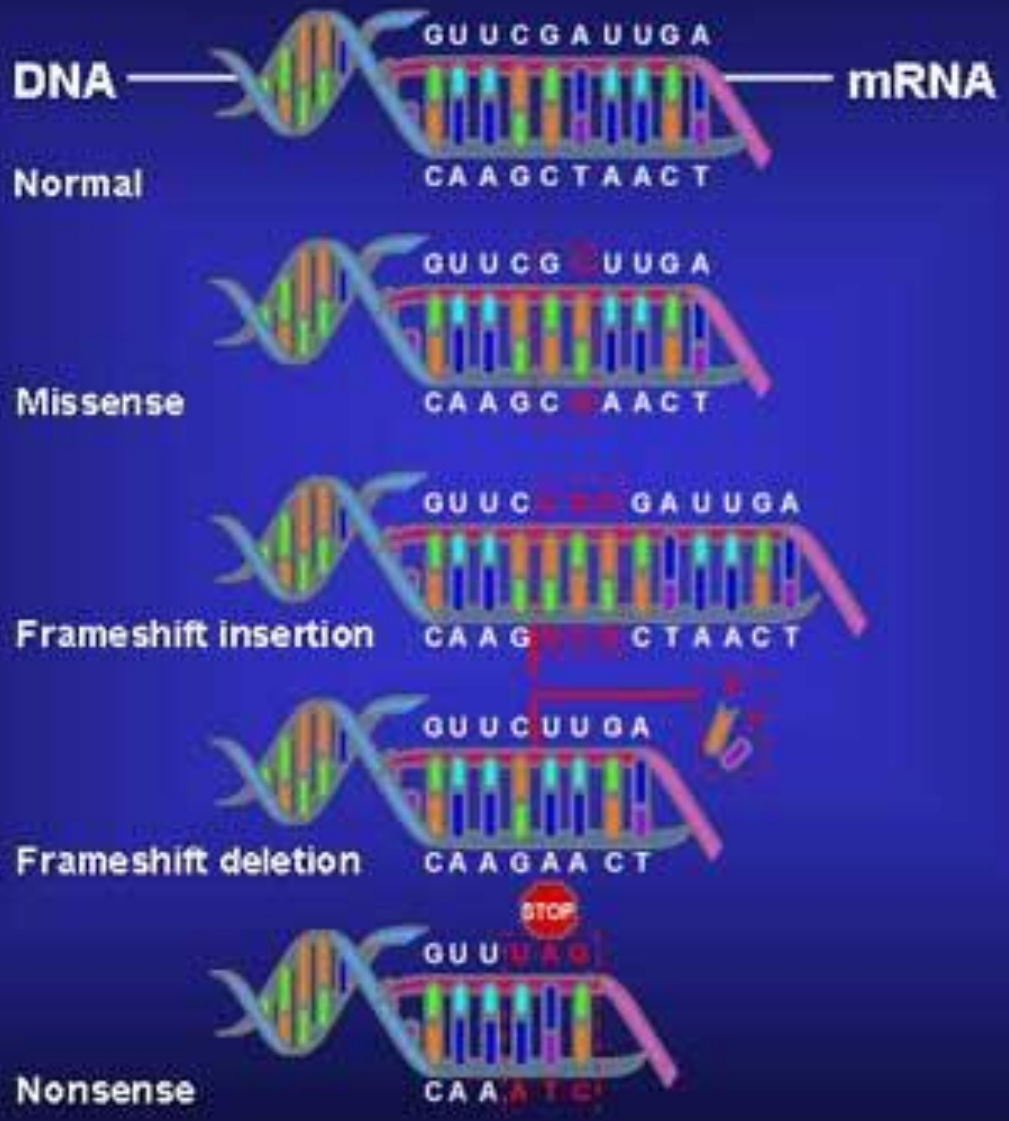
Point Mutation



	U	C	A	G
U	UUU = phe	UCU = ser	UAU = tyr	UGU = cys
	UUC = phe	UCC = ser	UAC = tyr	UGC = cys
	UUA = leu	UCA = ser	UAA = stop	UGA = stop
	UUG = leu	UCG = ser	UAG = stop	UGG = trp
C	CUU = leu	CCU = pro	CAU = his	CGU = arg
	CUC = leu	CCC = pro	CAC = his	CGC = arg
	CUA = leu	CCA = pro	CAA = gln	CGA = arg
	CUG = leu	CCG = pro	CAG = gln	CGG = arg
A	AUU = ile	ACU = thr	AAU = asn	AGU = ser
	AUC = ile	ACC = thr	AAC = asn	AGC = ser
	AUA = ile	ACA = thr	AAA = lys	AGA = arg
	AUG = met	ACG = thr	AAG = lys	AGG = arg
G	GUU = val	GCU = ala	GAU = asp	GGU = gly
	GUC = val	GCC = ala	GAC = asp	GGC = gly
	GUA = val	GCA = ala	GAA = glu	GGA = gly
	GUG = val	GCG = ala	GAG = glu	GGG = gly


No mutation
DNA
A T G
mRNA
U A C
tyrosine (normal protein)
Point mutations
A T A
U A U
tyrosine (normal protein)
A T C
U A G
stop (incomplete protein)
G T G
C A C
histidine (faulty protein)
Silent M.
Non Sense M.
Missense M.

Point Mutations

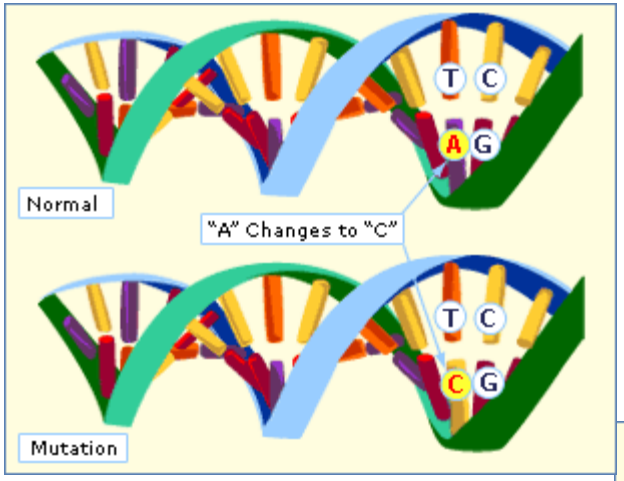


Chromosomal mutation

- Mutation associated with splitting and subsequent changes in the structure of the chromosomes

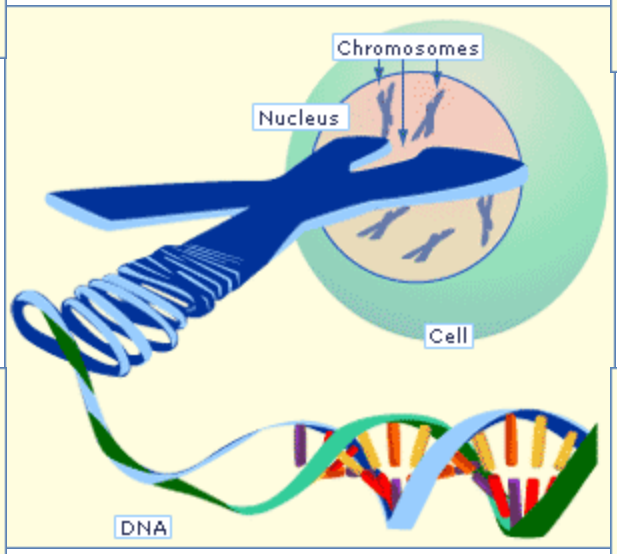
- The end of the split chromosomes may fuse to form structure again, but the new chromosomes are not always exactly what the used to be

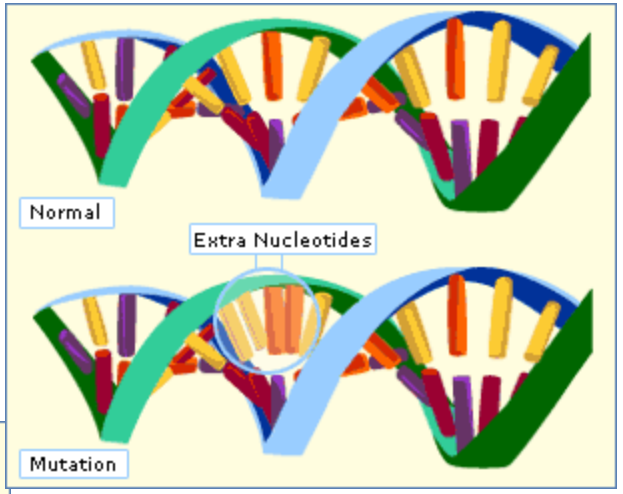
- The microscopic structures of chromosomes may be characterized by **deletion** or **deficiency** (loss of a chromosomal segment), **duplication** (doubling of a chromosomal segment), **inversion** (rearrangement of a group of genes in a chromosomal segment in a such a way that their order is reversed; rearrangement of genetic material in a chromosome results from loss of segment, its rotation by 180°, and fusion of the separated ends) and **translocation** (change in a position of a chromosome or more often exchange of segments between different chromosomes)



Point Mutation

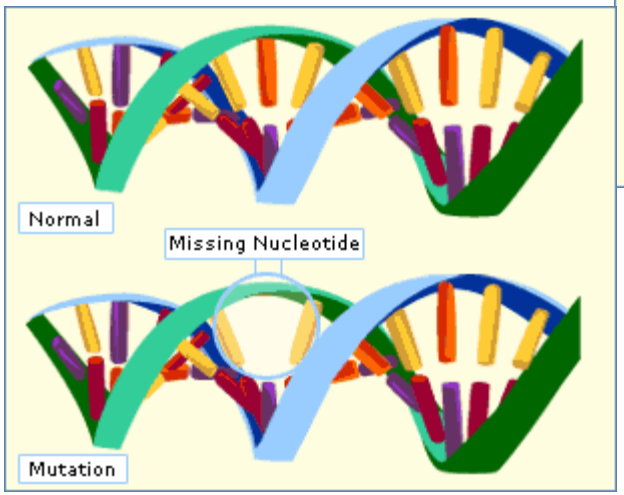
This is a single change in one of the four nucleotide bases. An" A " might change to a" C " for example.





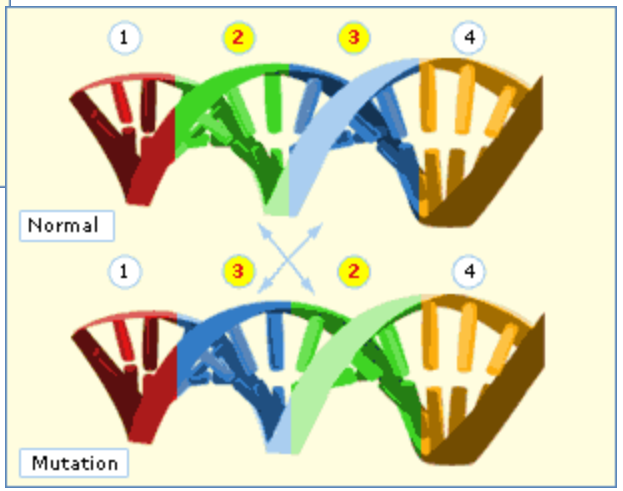
Insertion

Extra nucleotides are added to the sequence.



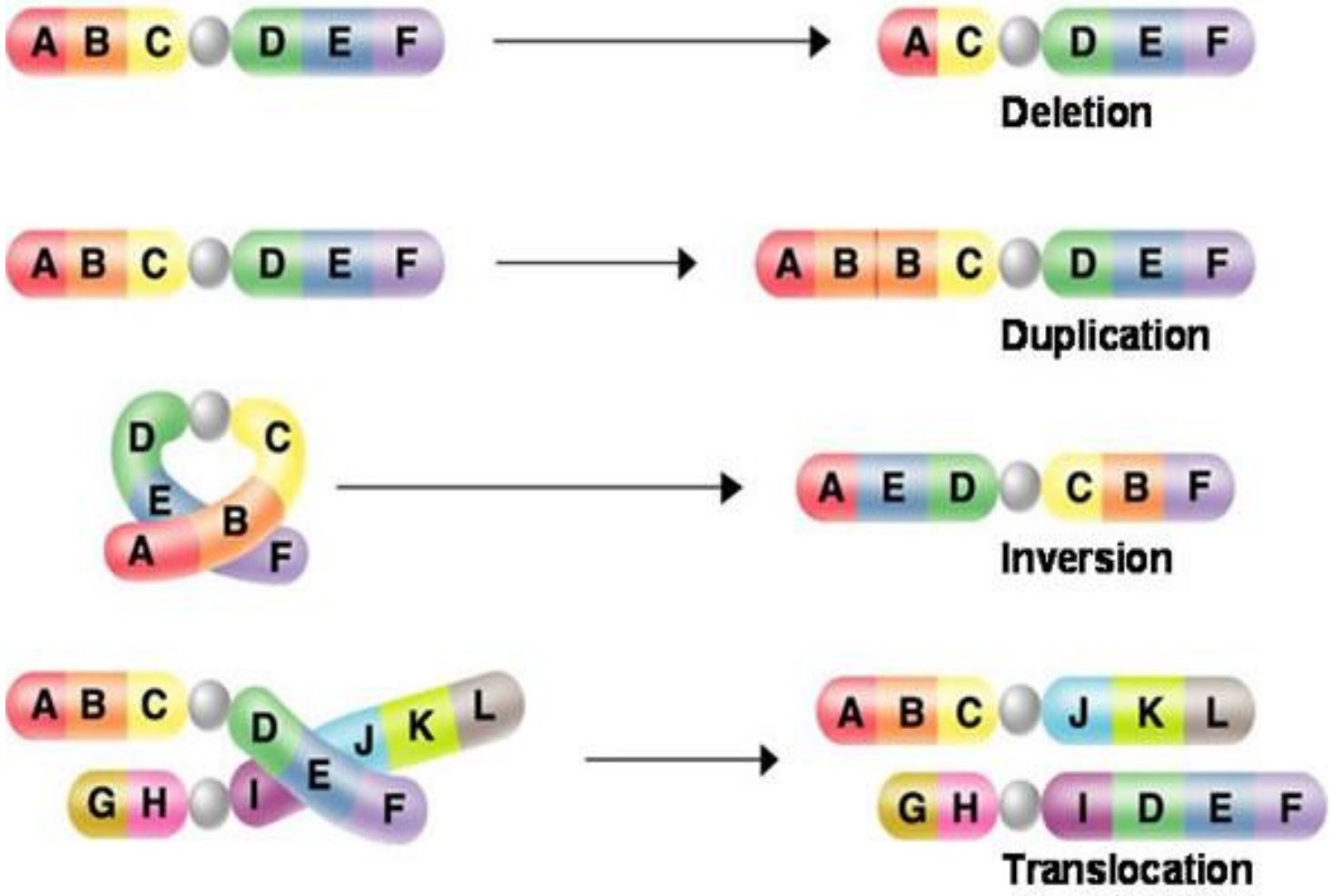
Deletion

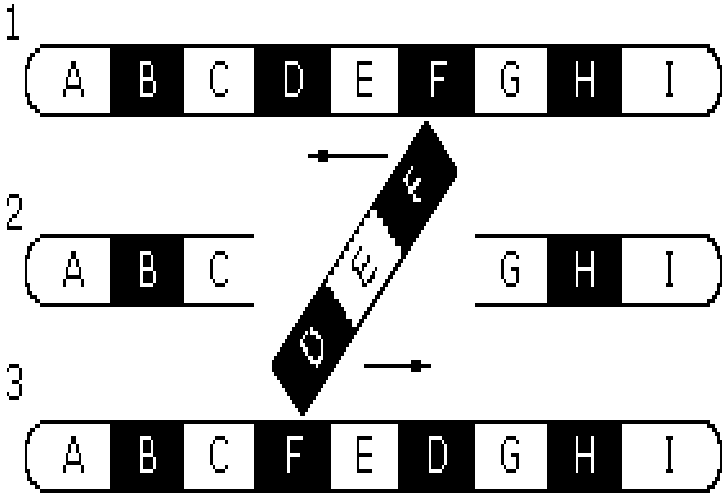
Part of the DNA sequence is missing.



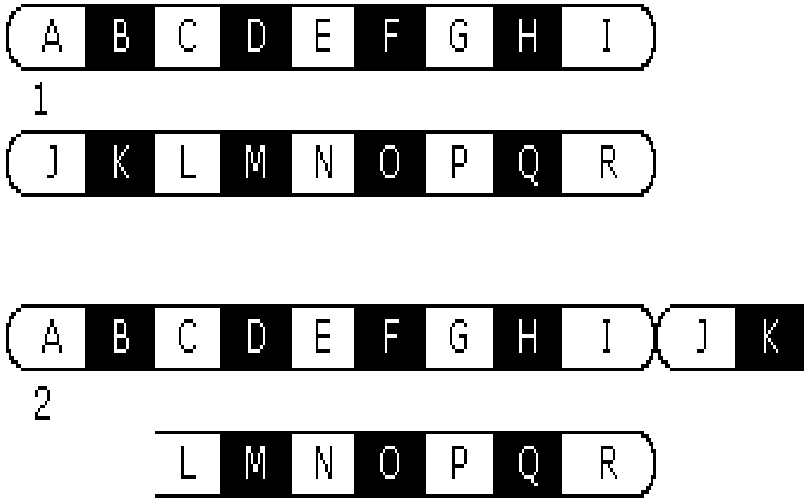
Inversion

A portion of the DNA sequence is reversed.


A B C D E F
A C D E F
Deletion
A B C D E F
A B B C D E F
Duplication
D C E B A F
A E D C B F
Inversion
A B C D J K L
G H I E F
A B C J K L
G H I D E F
Translocation



Inversion



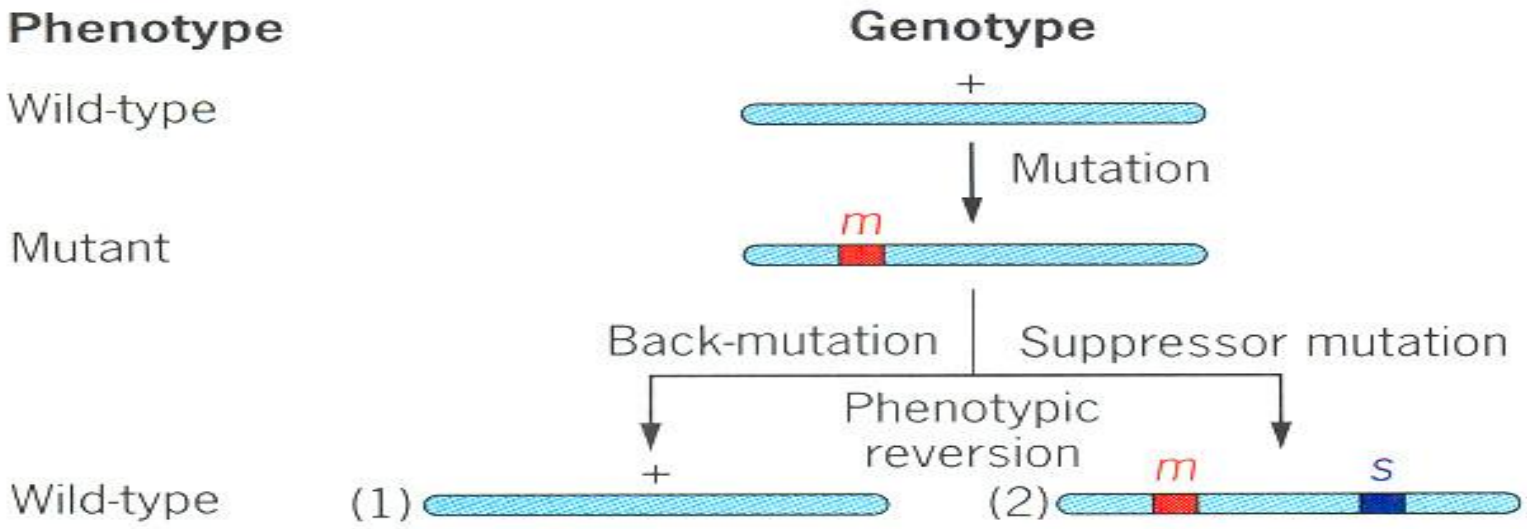
Translocation

Genome mutation

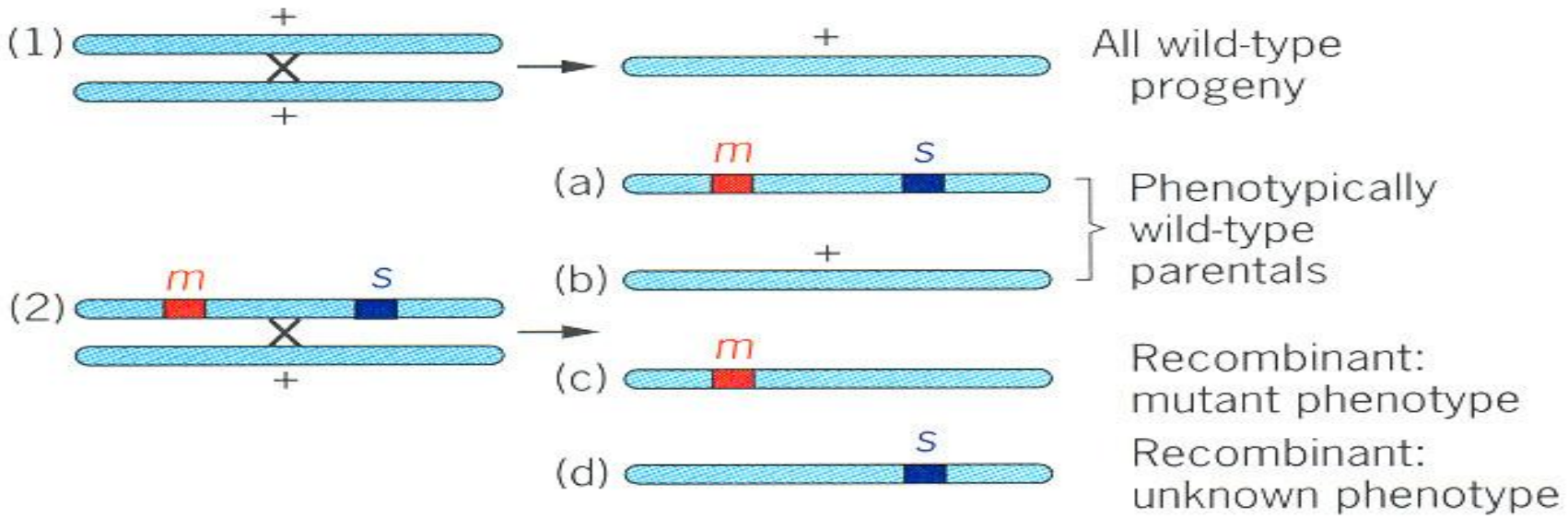
- Changes in sets of chromosomes

Remarks:

1. Breeders are more interested in gene mutation, because chromosomal rearrangement usually produce negative results, such as lower fertility of the offspring
2. Mutant are aften of great value for breeding as sources of new, previously unknown useful characters
3. Mutagenesis may be instrumental in obviating the technical difficulties arising in the crossing of such a small flowered crops such as milled



Backcross to wild-type:



Type of mutation

- Spontaneous (natural) mutation

1. Some have played an outstanding role in development of valuable crop cultivars and hybrids
2. Unfortunately, it can not form the basis of modern plant breeding due to its low frequency and difficulties in detection

- Induced mutation

Technique for inducing mutation

- ✓ Physical mutagens
- ✓ Chemical mutagens

Physical mutagens

1. **Various sources of ionizing radiations are explored, most often X and gamma rays, UV radiation, fast and slow neutron, alpha ray, beta ray**
2. **Radioactive isotopes P-32 and S-35 are not convenient for use due to the storage and application difficulties**
3. **The usual sources of gamma rays in laboratories are radioactive cobalt (Co-60) and Cesium (Cs-137) placed in cobalt bomb**

Physical mutagens

4. The object can be irradiated in two ways:

- ✓ With an aid of a powerful source of a short-duration gamma rays for short duration radiation. Need special units for irradiating living object
- ✓ A much weaker radiation but operating continuously (gamma field).

5. the dosage must be varied depending not only on the plant species whose seeds/organs are irradiated, but also on many other factors
6. plant must be irradiated heavily enough to ensure as many inherited changes as possible but without seriously affecting the germination, growth and fertility of plant directly emerging from the irradiated seeds or vegetative organs (*critical radiation dose*: dosage which strong enough to assure many mutation not yet so strong as to kill plants*)*

Chemical mutagens

- ✓ Mutagenic substances belonging to different classes of chemical compounds, such as ethylene imine, diethyl sulfate, dimethyl sulfate, N-nitrosoethyl urea, N-nitrosomethyl urea, methal sulfonate, diepoxy butane, ethyleneoxide
- ✓ Most are highly toxic, usually result in point mutations
- ✓ Use in solution in the concentration ranging from tenth – hundredths even thousandths of percent
- ✓ Many chemical mutagens are much more effective than physical one. If irradiation of crops produces 10 – 15% of viable inherited changes, chemical mutants do the same at a rate of 30 to 60%
- ✓ They often exert more specific and finely tuned action on the cell

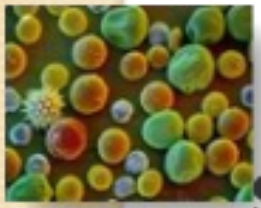
Chemical mutagens

- ✓ Some substances (supermutagen) are capable of causing inherited changes in plants at a rate up 100%
- ✓ Chemical mutagens aim at the most vulnerable spot of a living organism (DNA) to induce changes in nucleotides and alter the genetic information (Sometimes causes specific mutation)
- ✓ It provides a powerful tool to induce desire changes in a trait

Part of the plant to be treated

- Seeds, pollen grains, buds/cuttings or complete plant can be used for mutagenesis.

It depends on whether the crop is sexually or asexually propagated & type of mutagen to be used.



Sexually propagated crops :

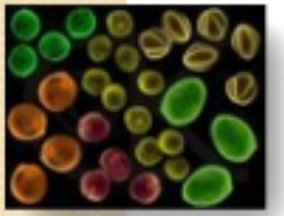
Seeds are commonly used because seed can tolerate extreme environmental conditions.

Use of mutations in sexually reproduced crops

- ✓ More valuable in self than cross pollinated. The probability of producing desirable mutations and genetic variability is theoretically higher

- ➢ Seeds
- ➢ Very young seedling

- Pollen grains are also infrequently used because :-



i. They are difficult to collect in large quantities .

ii. Hand pollination(with treated pollens) is rather difficult.

iii. Survival percentage of pollens is relatively low.

Note:- Pollen grains are the only plant part which can be treated with ultra violet rays.

Clonal crops : Buds/cuttings are used for mutagenesis.

Use of mutations in asexually produced crops

- ✓ It has been much easier and quicker to obtain variant plant types
- ✓ Specific location of the mutation event (segmental chimera) becomes important.
- ✓ The mutant must be in meristematic tissue that will produce faithfully through cutting or other vegetative means

- ➢ Bud
- ➢ Scion
- ➢ Cutting
- ➢ Tuber
- ➢ bulbs

Dose and duration of the mutagen

- The usefulness of a mutagen and its efficiency depends on the mutagenic agent employed as well as on specific characteristics of the biological system to be treated.

- An optimum dose is the one which produce the maximum frequency of mutation and causes minimum killing.

  LD_{50} dose should be optimum.

Description of generations

- **M1** Plants obtained from treated seeds/cuttings or from seeds obtained after pollination with treated pollens are called M1 plants

Treated seed/buds/cuttings

Pollination with treated pollen

M1 Plants

Mutation breeding scheme for seed propagated crop

- ✓ Mutagenic application
- ✓ Growing the plants (M1 generation)
- ✓ Identification of induced mutation, seed harvest from mutated plants (M2)
- ✓ Continue the identification and selection of induced mutation (M3)
- ✓ First agronomic evaluation. Propagation of promising lines (M4)
- ✓ Multilocation trials of stable mutant and recombinant lines (M5 – M8)
- ✓ Official testing and releasing of mutant (M9)

Description of generations

- **M1** Plants obtained from treated seeds/cuttings or from seeds obtained after pollination with treated pollens are called M1 plants

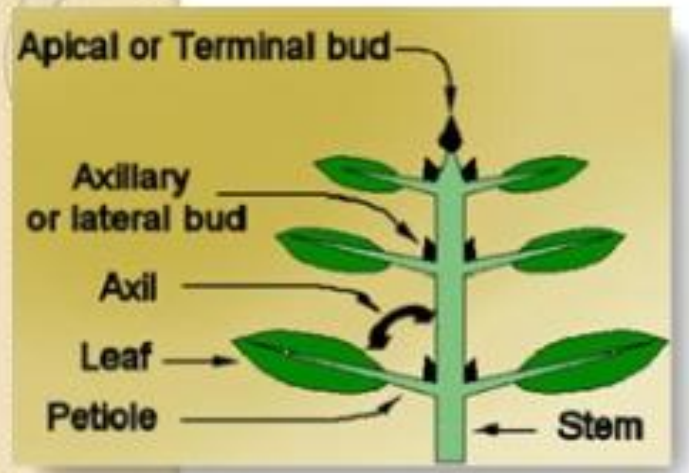
M1 Plants

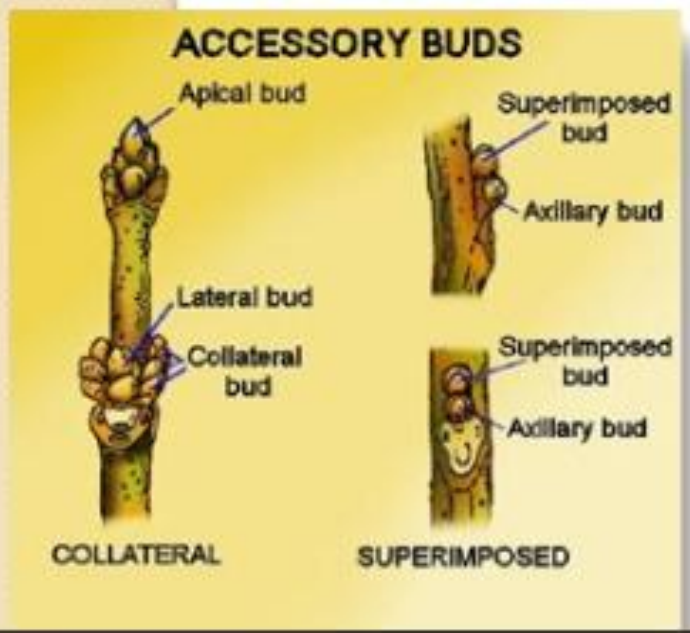
- Large no. of plants are grown
- Grown in wider spacing.
- Dominant mutations are recorded if any (generally mutations are recessive and do not express in M1)
- Chlorophyll sectors and fertility is recorded.
- M1 plants are selfed and their seed is harvested separately.

Mutation breeding scheme for vegetative propagated crop

- ✓ Mutagenic application
- ✓ Cutting back the M1V1 shoot, bud grafting, or in vitro propagation via axillary buds
- ✓ Isolation of induced somatic mutation, establishment of clones, cutting back of non-mutant shoots from chimeric plants (M1V2)
- ✓ Further isolation of somatic mutations, vegetative propagation of mutant plant (in vivo or in vitro), preliminary evaluation of mutants (M1V3)
- ✓ Evaluation of mutant clone performance, assesing segregation from mutant crosses and reselection of desired recombinants. Released of improved mutant (M2V4)

Procedure for Vegetative propagated crops





❖In apical buds, axillary buds and adventitious buds, there are two functional layers, outer layer and inner layer. When the changes occur in entire inner or outer layer, it is also known as ***Periclinal Chimera*** and when only a part of inner or outer layer is altered, it is called ***Sectorial Chimera.***

Chimera ?

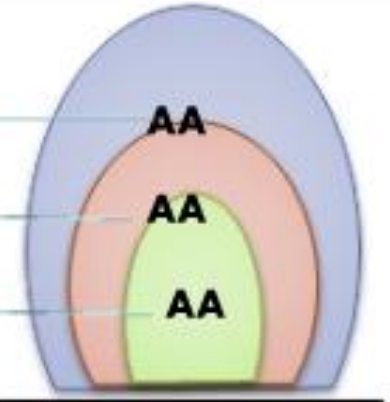
- Vegetative mutations are expressed in the form of chimeras.
- Periclinal chimera (whole of inner and outer layer)
- Sectorial chimera(part of inner/outer layer)
- Seed propagated crop-inner layer important…seed formation

Chimera - a plant or plant part composed of genetically different layers

Chimera ?

Three functional layers

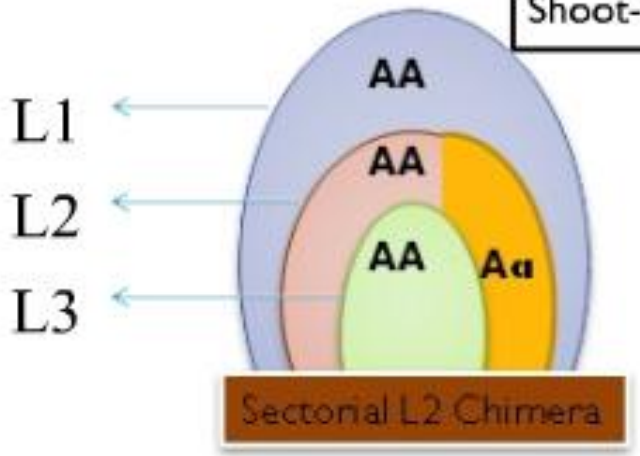
Produces Epidermis	L1
Produces leaf mesophyll and gametes.	L2
Produces rest of plant body	L3

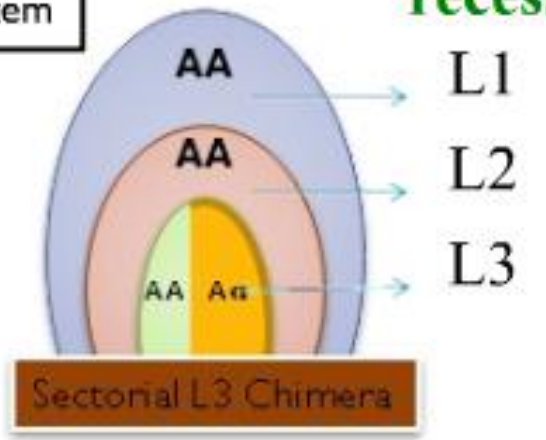


Shoot-Tip Meristem

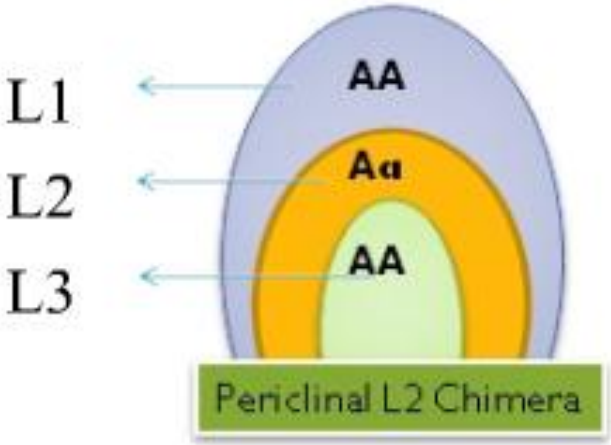
Sectorial and Periclinal Chimeras produced by mutagenesis. The Mutant allele (a) is recessive.

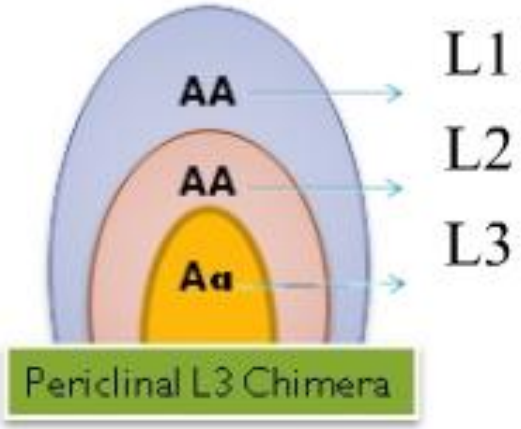
When part/segment of the L1, L2 or L3 is affected, it is called SECTORIAL CHIMERA



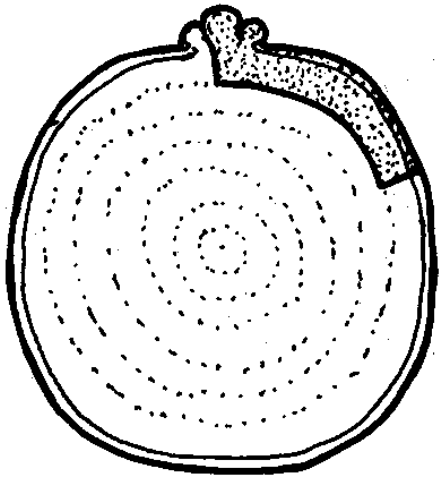


When whole of the L1, L2 or L3 is affected, it is called PERICLINAL CHIMERA

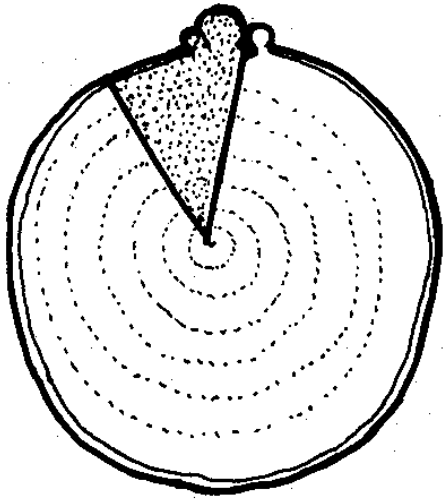




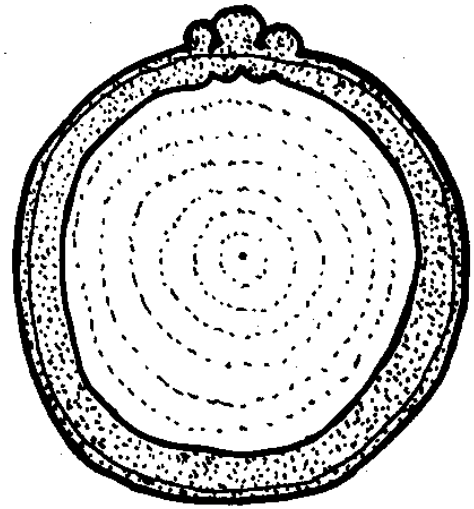
Types of Chimeras



Mericlinal



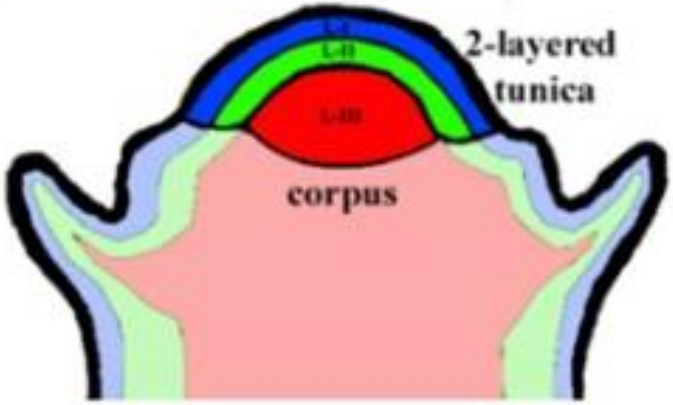
Sectorial

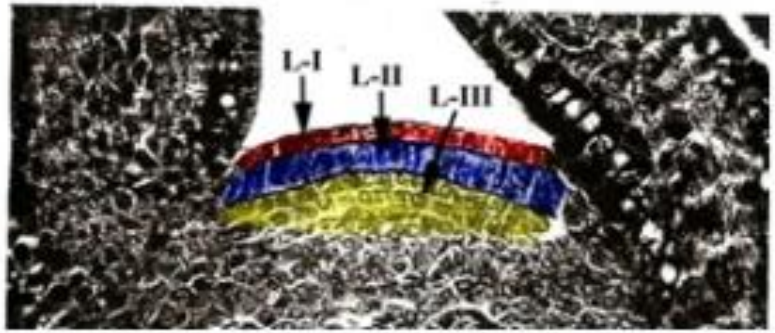


Periclinal

Chimera ?

Chimera - a plant or plant part composed of genetically different layers





The most common example is a "variegated" plant where different regions of the leaf are yellow or white due to the lack of chlorophyll synthesis, i.e. these are chlorophyll mutants. However, there are many kinds of chimeras. Thornless blackberries are chimeras where the L-I epidermis lacks the ability to produce thorns. Some fruits have sweet and sour regions of flesh, which may be a chimera.

Alöe mitriformis variegata

Variegated Geranium

Acer platanoides

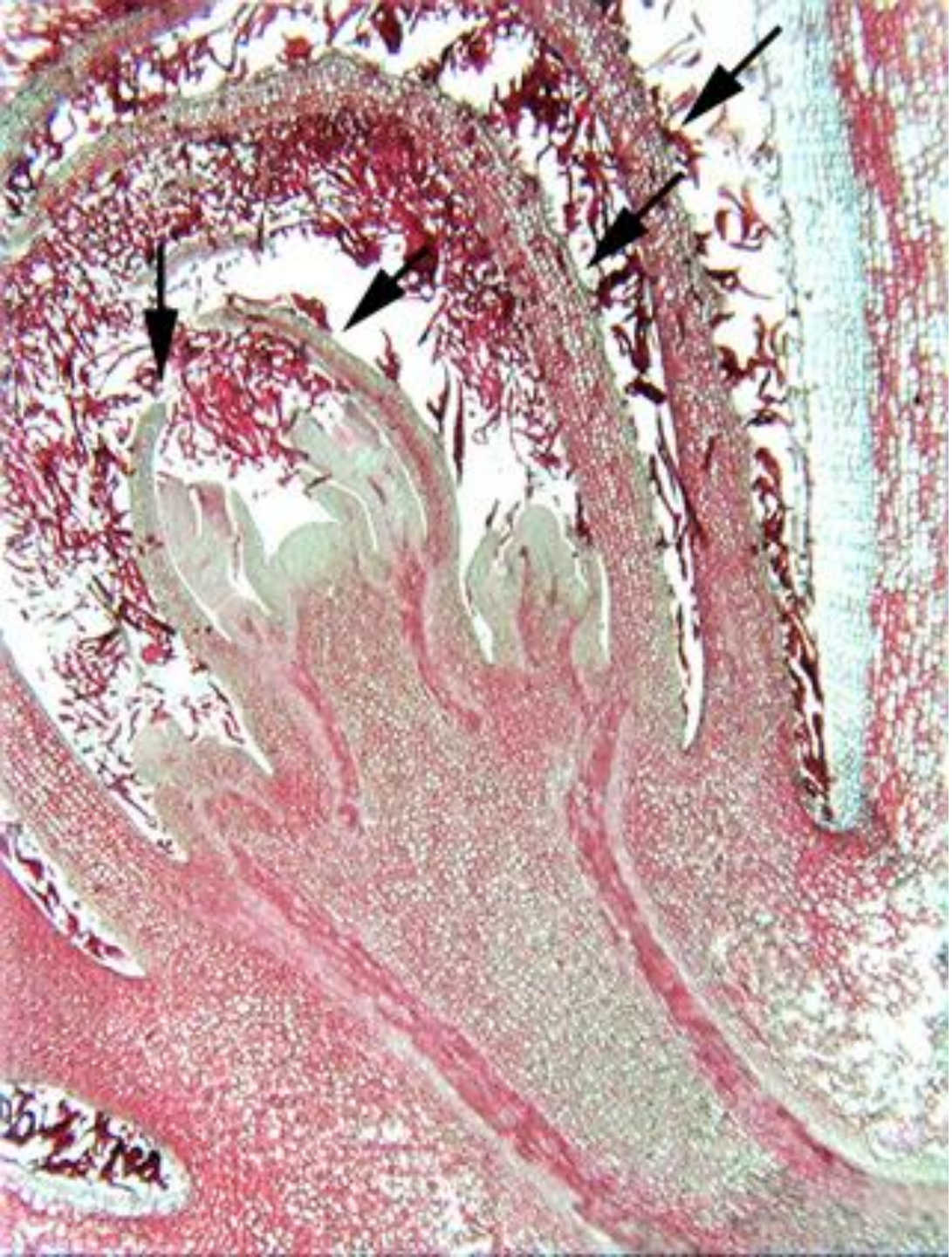
"Drummondii" (Family: Aceraceae)
Common name: Variegated Norway maple

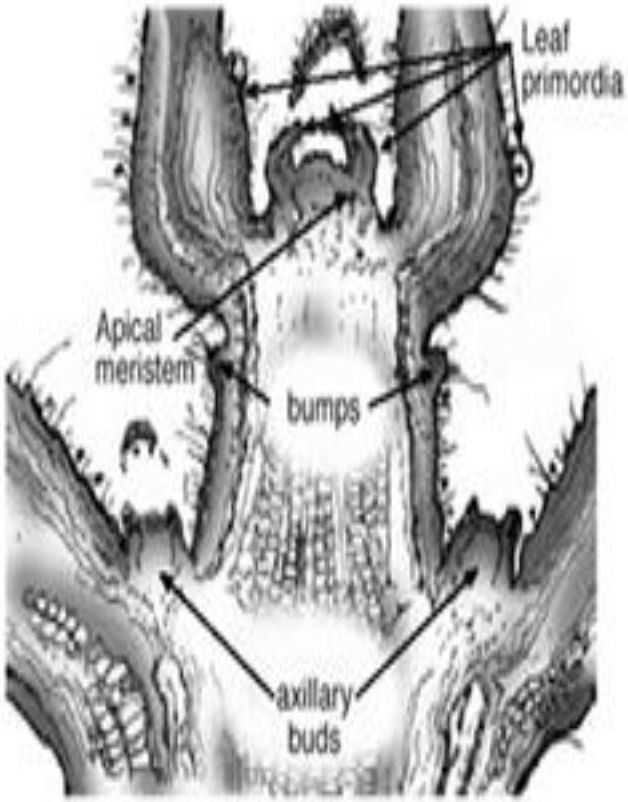
Chimeras

Albino peach shoot

Partial reversion of Red Delicious

Sectorial chimera (mutation)




Leaf primordia
Apical meristem
bumps
axillary buds

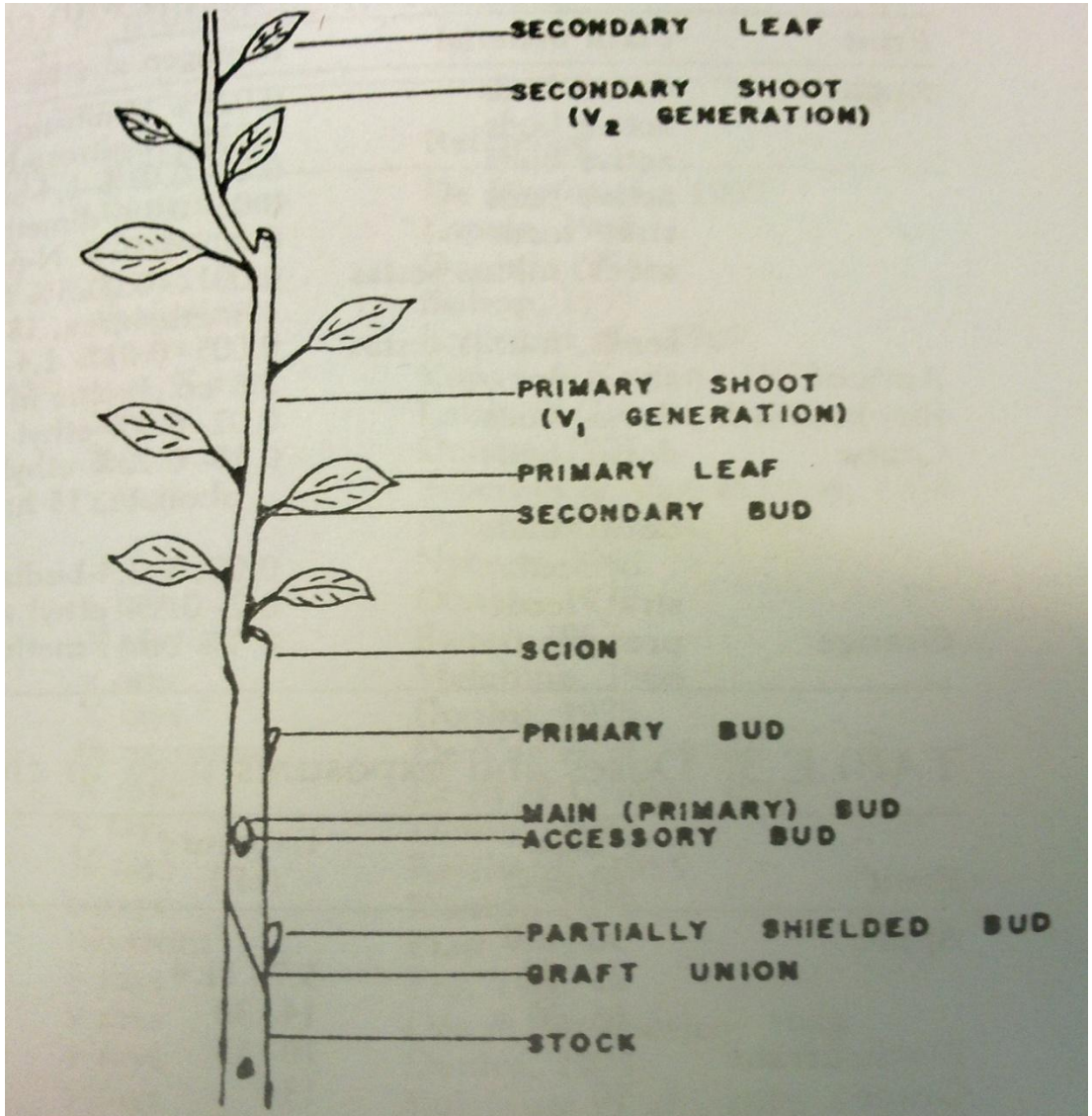
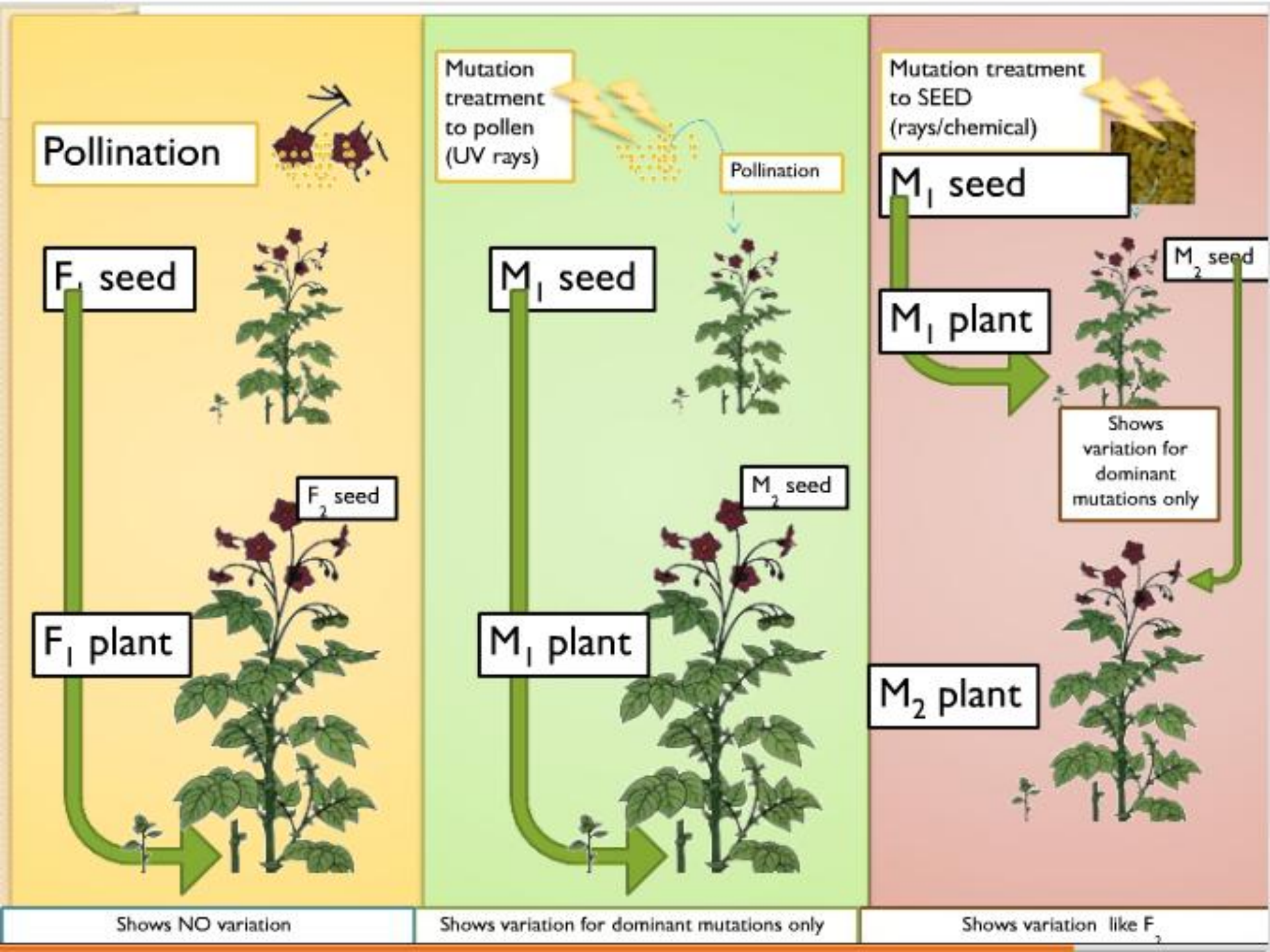


FIG. 2. Schematic drawing of an irradiated scion with primary and secondary buds, leaves, and shoots.


Pollination
F1 seed
F1 plant
F2 seed
Mutation treatment to pollen (UV rays)
Pollination
M1 seed
M1 plant
M2 seed
Mutation treatment to SEED (rays/chemical)
M1 seed
M1 plant
M2 seed
Shows variation for dominant mutations only
M2 plant
Shows NO variation
Shows variation for dominant mutations only
Shows variation like F2

Handling of mutagenic population

Treatment of seeds & vegetative propagules commonly produce **chimeras.**

Shoot tip meristem has three layers :-

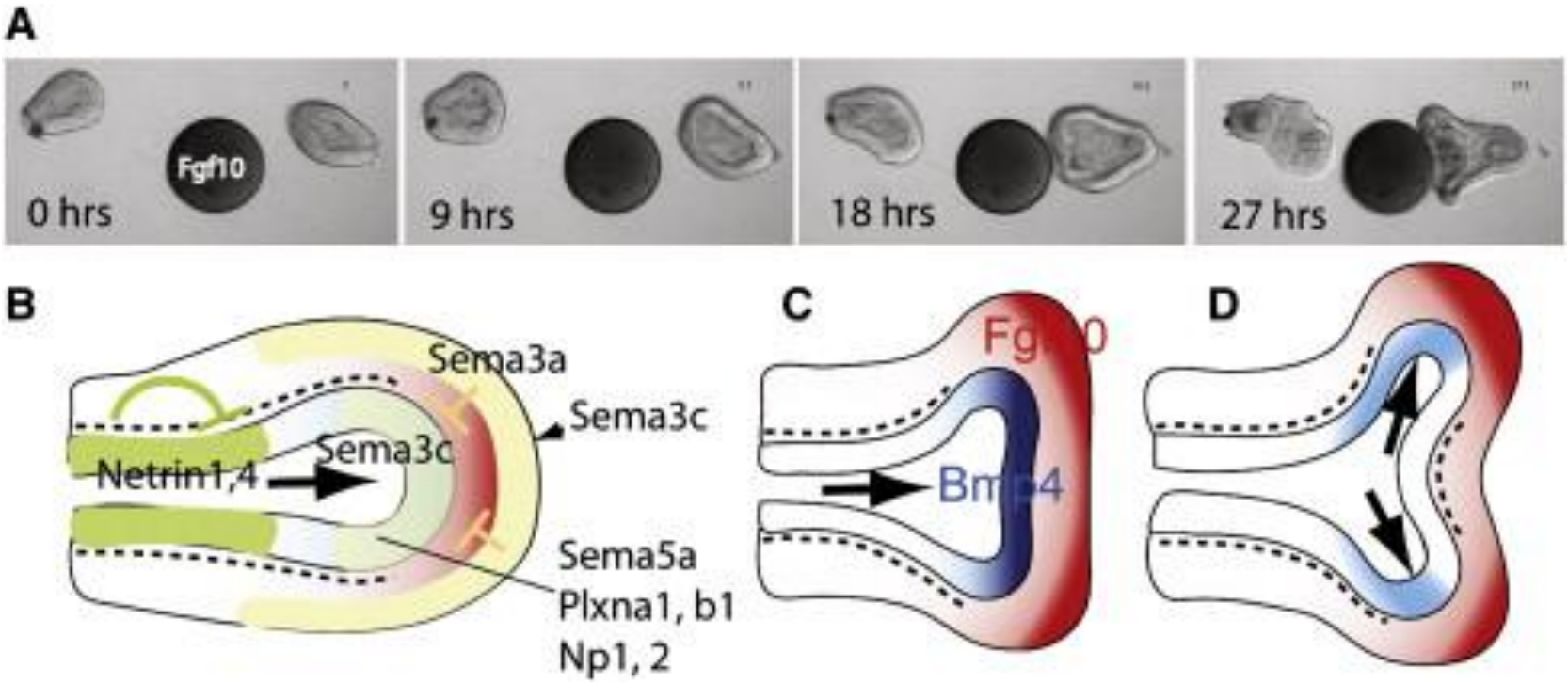
- **L_1 :-** Give rise to epidermis.
- **L_2 :-** Part of leaf mesophyll & gametes.
- **L_3 :-** Yield the rest of plant.

Periclinal chimera :- When the whole of **L_1 , L_2** or **L_3** layer is affected.

Sectorial chimera :- When only a part of **L_1 , L_2** or **L_3** layer is affected .

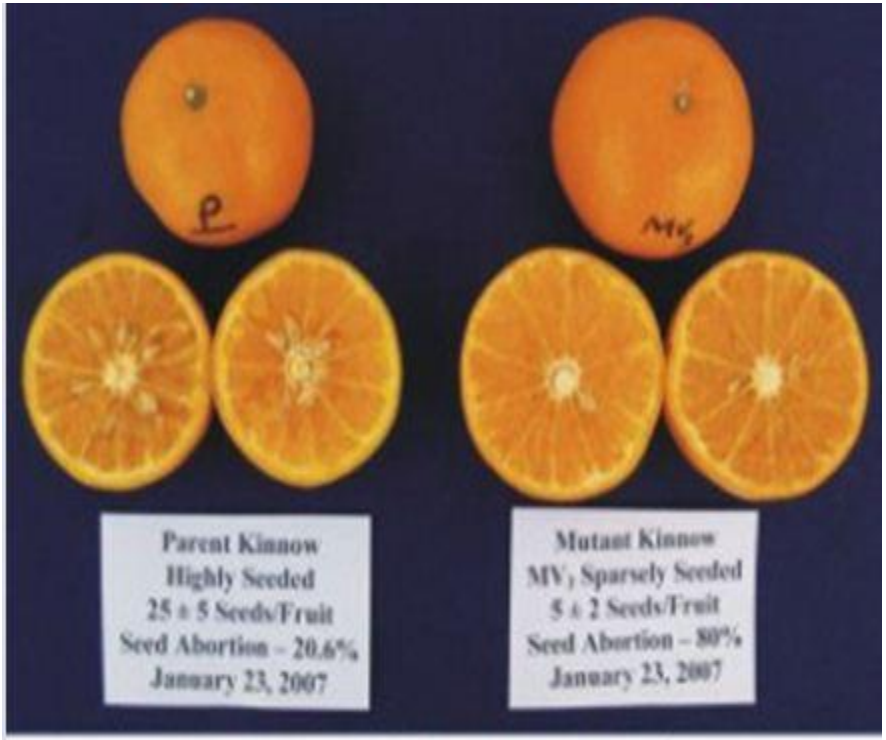
- In sexually reproducing species L_2 chimera will be transmitted to the next generation.

- In sexually reproducing crops mutation breeding utilize both recessive & dominant mutations.


A
Fgf10
0 hrs
9 hrs
18 hrs
27 hrs
B
Sema3a
Sema3c
Sema3c
Netrin1,4
Sema5a
Plxna1, b1
Np1, 2
C
D

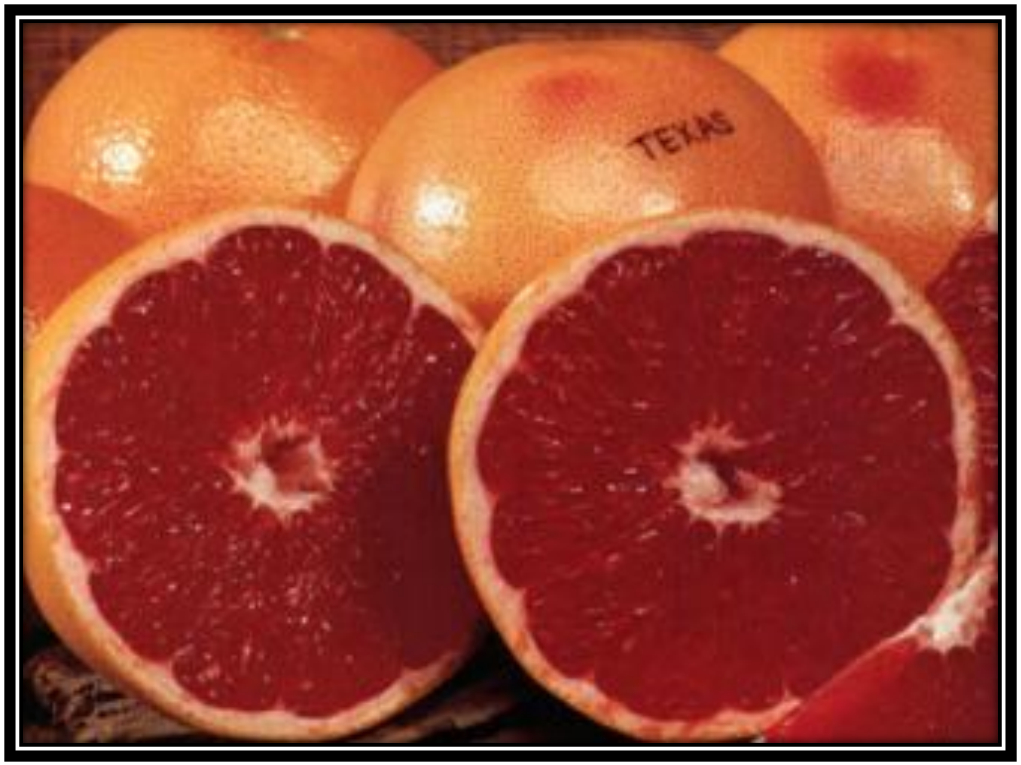
Schedules for propagation and selection of mutagen treated fruit plants

Year	Season	Procedure
		Schedule A. (Original selection in V2)
1	Winter	Irradiation, grafting in a greenhouse (500-1000 grafting). V1 generation
1	Summer	Budding, three to five buds from each primary shoot
2	Summer	First selection in V2. Budding, 10 buds from each selection
3	Autumn	Observation and measurement in V3. Second selection.
4	Spring	Planting potential mutants in an orchard for the first test.
5-9-11		Evaluation of trees and fruits, analysis of data selection
10-12		Propagation of promising mutants for the second orchard test.
		Schedule B. (Original selection in V1)
1	Spring	Irradiation, grafting in the nursery (×1000 grafting). V1 generation
1	Summer	First selection in V1. Budding, 10 buds from each selection
2a-9a-11a		Continue as in *schedule A* (years 3 to 10 or 12)
2b	Spring	Pruning back all V1 shoots
2b	Summer	Reduction V2 shoots to one per stock. First selection in V2. Budding, 10 buds from each selection
3b-10b-12b		Continue as in *schedule A* (years 3 to 10 or 12)



Low seeded Kinnow mandarin

- Seedless Kinnow is the demand of local juice industry and the foreign markets. A sparse seeded (5 $\pm$ 3 seeds/fruit) mutant Kinnow has been developed as a result of induced mutation with gamma irradiation of dormant bud of high seeded (25 $\pm$ 5 seeds/fruit) parent Kinnow. The conventional scion/stock graft techniques were used for the propagation of mutant. The material has been taken upto mV_5 stage of vegetative propagations, with the confirmation of continuity of the induced character. The rootstocks of *Citrus jambhiri* are being grafted from scions of mV_5 and provided to farmers for general cultivation.

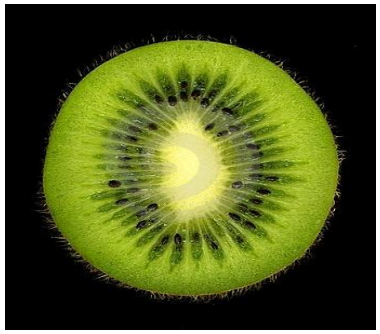


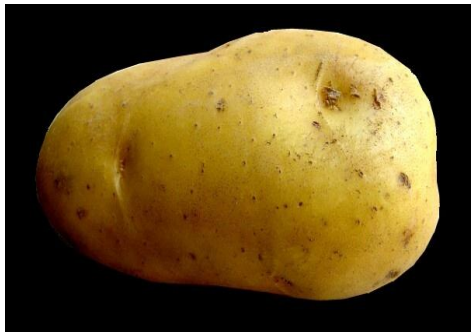
- The **Rio Star® Grapefruit**, a product of radiation-induced mutations, now accounts for three quarters of all grapefruit trees grown in Texas. The mutant strain was developed by Richard A. Hensz, a Texas horticulturalist and director of the Texas A&I University Citrus Center in Weslaco, Texas. Working at the Brookhaven National Laboratory in Long Island, Hensz began irradiating seeds from the famous **Ruby Red grapefruit** using X-rays in 1963. In 1976, he produced a new strain that was resistant to cold temperatures. Grapefruit crops had been devastated by severe freezes in the past; when another hit in December of 1983, the Rio Star® trees were spared. The Citrus Center began giving away Rio Star® seeds to farmers in 1984. The sweet, dark-red fruit quickly became popular among growers and consumers.

Gamma Field at the Institute of Radiation Breeding in Japan

- The gamma field encompassed 12.8 acres of land that Brookhaven scientists used to experiment on more than 300 plant species. In 1959, the researchers observed that radiation from the gamma field had injured nearby trees. The finding prompted them to create a 'gamma forest,' which examined the effects of gamma rays on an entire ecosystem. The gamma greenhouse was a concrete structure with a lead cap surrounded by earthen embankments. Cobalt-60 was raised from a shielded receptacle in the floor. The 'hot cell' was a small (2 meter) shielded chamber. A radiation source was lowered from the ceiling. Plants were placed on removable shelves that could be accessed through a set of sliding doors coated with lead. In the gamma pool, radioactive materials were lowered into the water to shower plant specimens placed at the bottom.

polyploidy







Polyploidy = the addition of one or more complete sets of chromosomes to the original set.

two copies of each autosome = **diploid**
four copies of each autosome = **tetraploid**
six copies of each autosome = **hexaploid**

Organisms with an odd number of autosomes, e.g., the domestic banana plant (*Musa acuminata*), cannot undergo meiosis or reproduce sexually.

Musa barbisiana (diploid)

Musa acuminata (triploid)

Monoploidy

…a haploid of a diploid is monoploid,

…has one chromosome set.

The gametes of diploids are haploid, those of tertraploids are diploid, those of hexaploid are triploid, and so on.

Monoploid Applications

- monoploid plants can be created by culturing pollen grains (n = 1),
  - the population of haploid organisms is then screened for favorable traits,
  - the plants are then treated with colchicine which generates a 2n plant homozygous for the favorable traits.

Two main types of polyploidy:
autopolyploidy (**genome doubling**) = the multiplication of one basic set of chromosomes
allopolyploidy = the combination of genetically distinct, but similar chromosome sets.

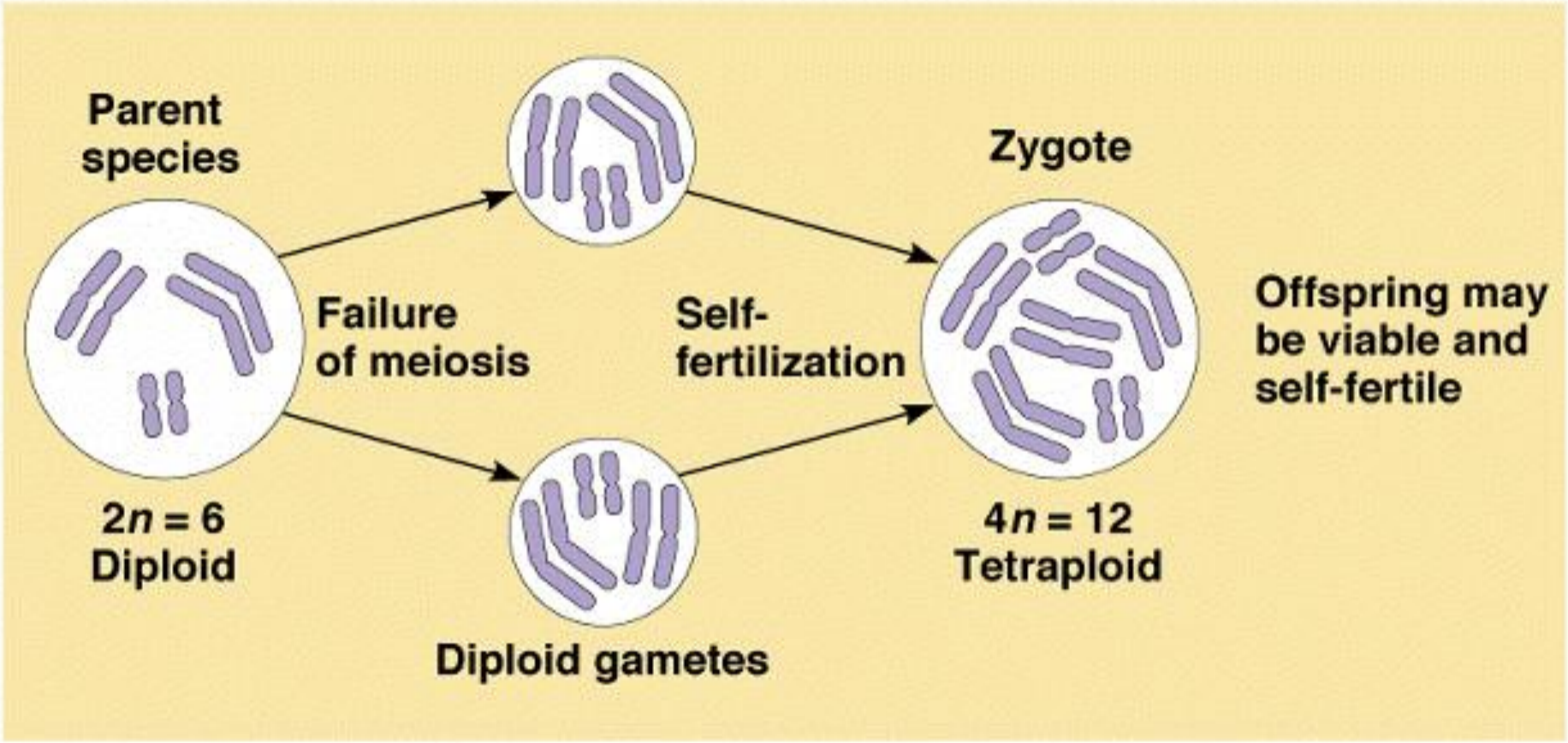
Autopolyploids are derived from within a single species; allopolyploids arise via hybridization between two species.

Autopolyploidy

...polyploidy resulting from the replication of one or more sets of chromosomes,

…the additional set of chromosomes is identical to the normal haploid complement of that species.

autopolyploidy



Autopolyploidy

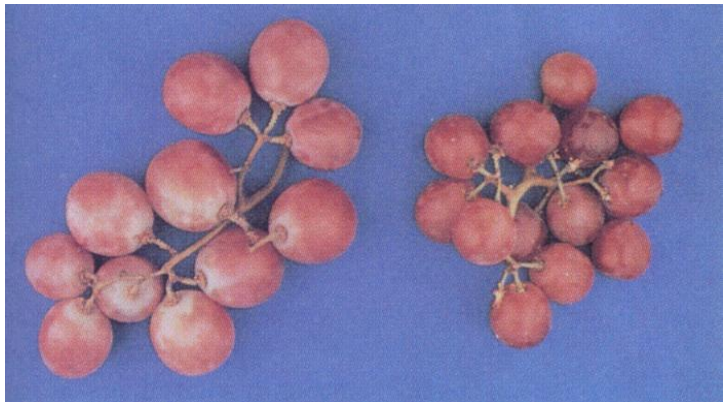
…can be induced by treating cells with the drug colchicine,

colchicine: is a alkaloid derivative from the autumn crocus (*Crocus veneris*),

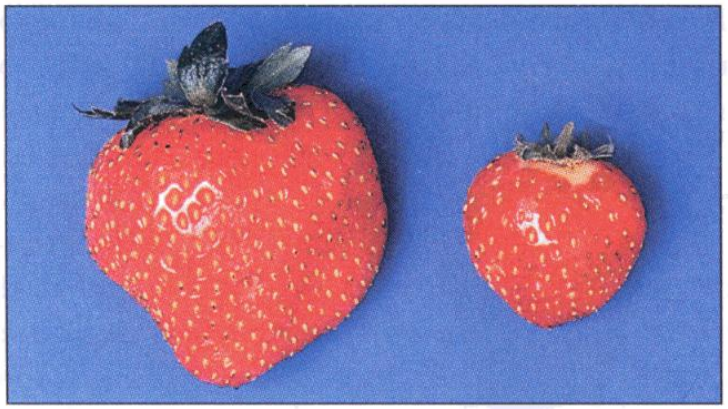
...inhibits microtubule polymerization, and thus inhibits the separation of chromosomes during meiosis.

Autopolyploidy Applications

- Treating a plant with colchicine often produces autopolyploidy, resulting in plants with larger flowers and/or fruit,

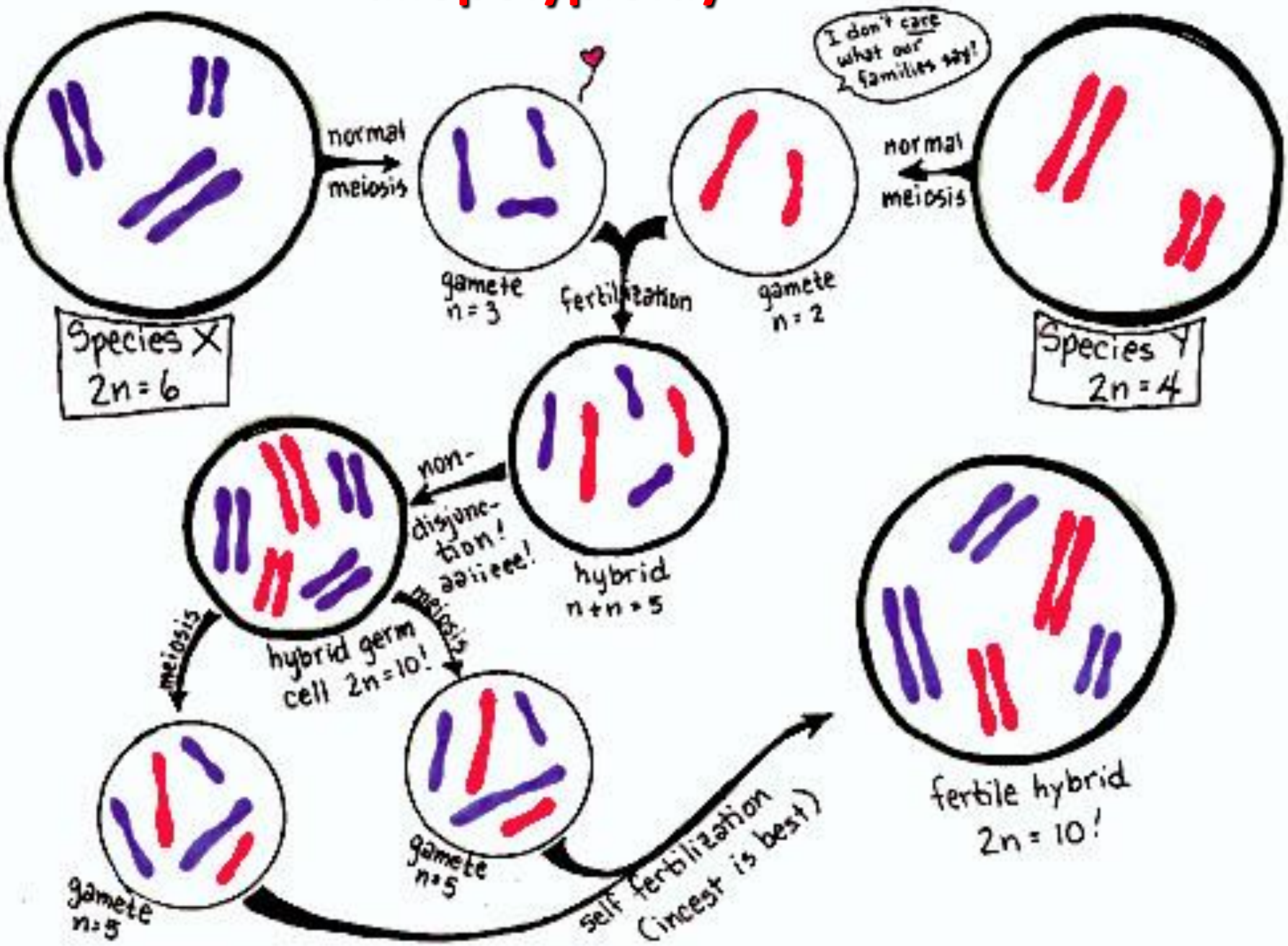


4n *2n*



8n *2n*

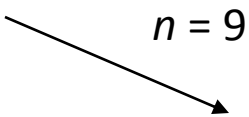
allopolyploidy



Allopolyploidy Applications

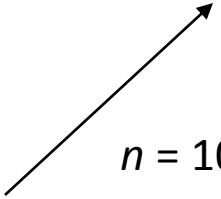
B. oleracea (cabbage, cauliflower, Brocolli, kale, etc.)

2*n* = 18



n = 9

B. napas (Oil rape, canola oil)



n = 10

B. campestris (turnip, turnip rape)

2*n* = 20

4n x 2n = 3n?

- The creation of triploids can be accomplished by crossing a tetraploid with a diploid,

- Most triploid individuals are sterile.

***Triticum urartu* (AA) × *Aegilops speltoides* (BB)**

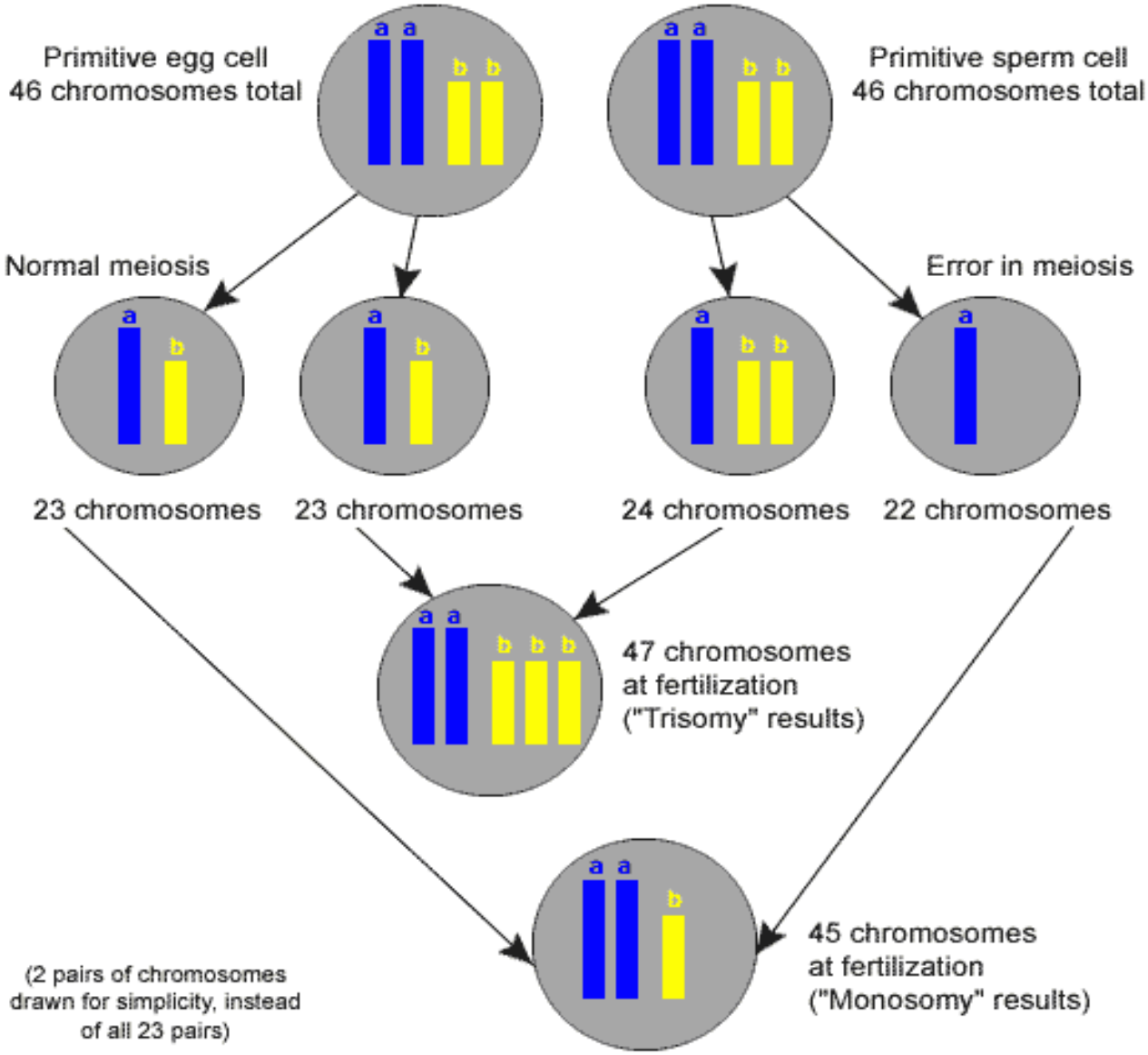
↓

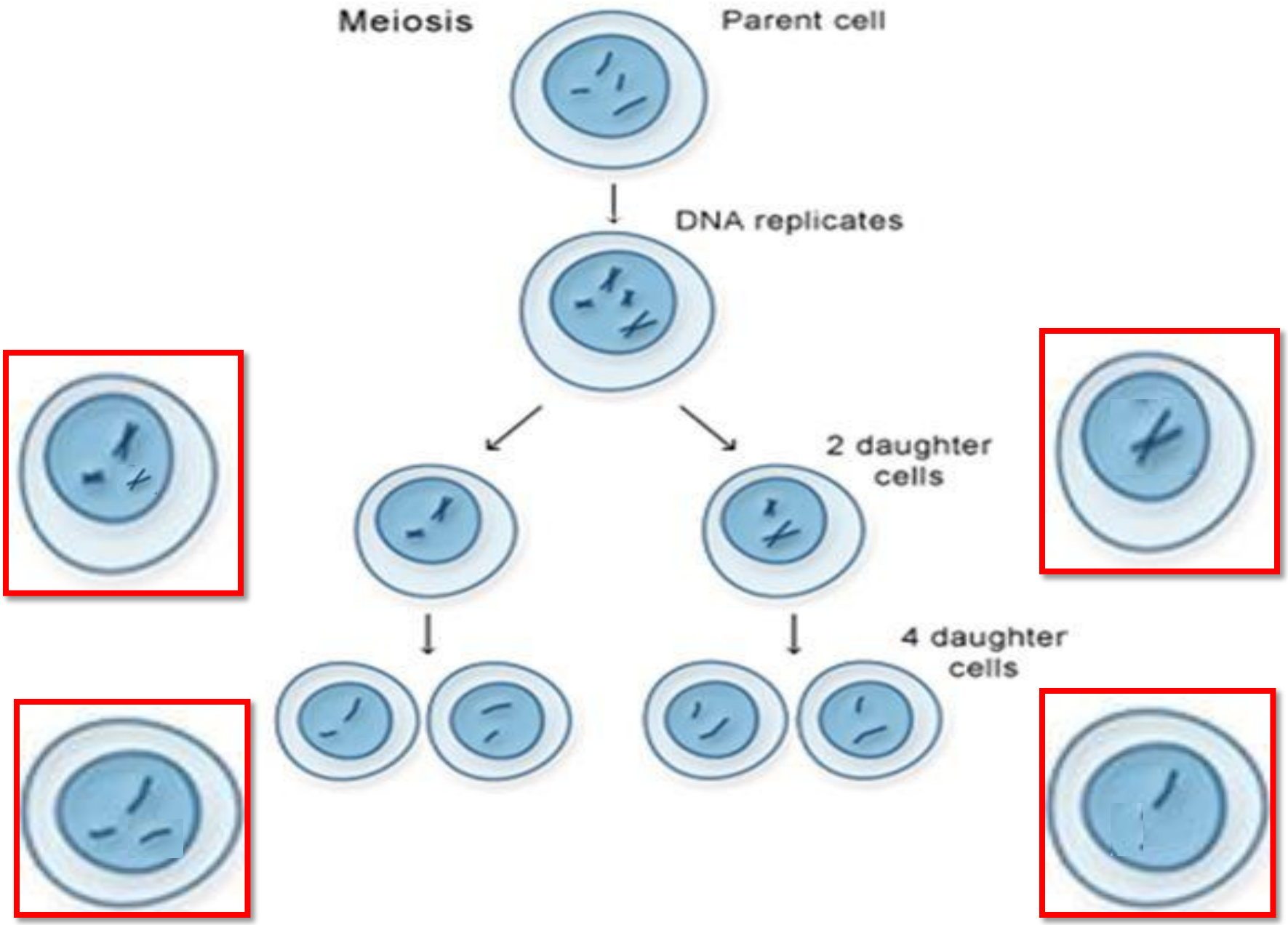
***T. turgidum* (AABB) × *T. tauschii* (DD)**

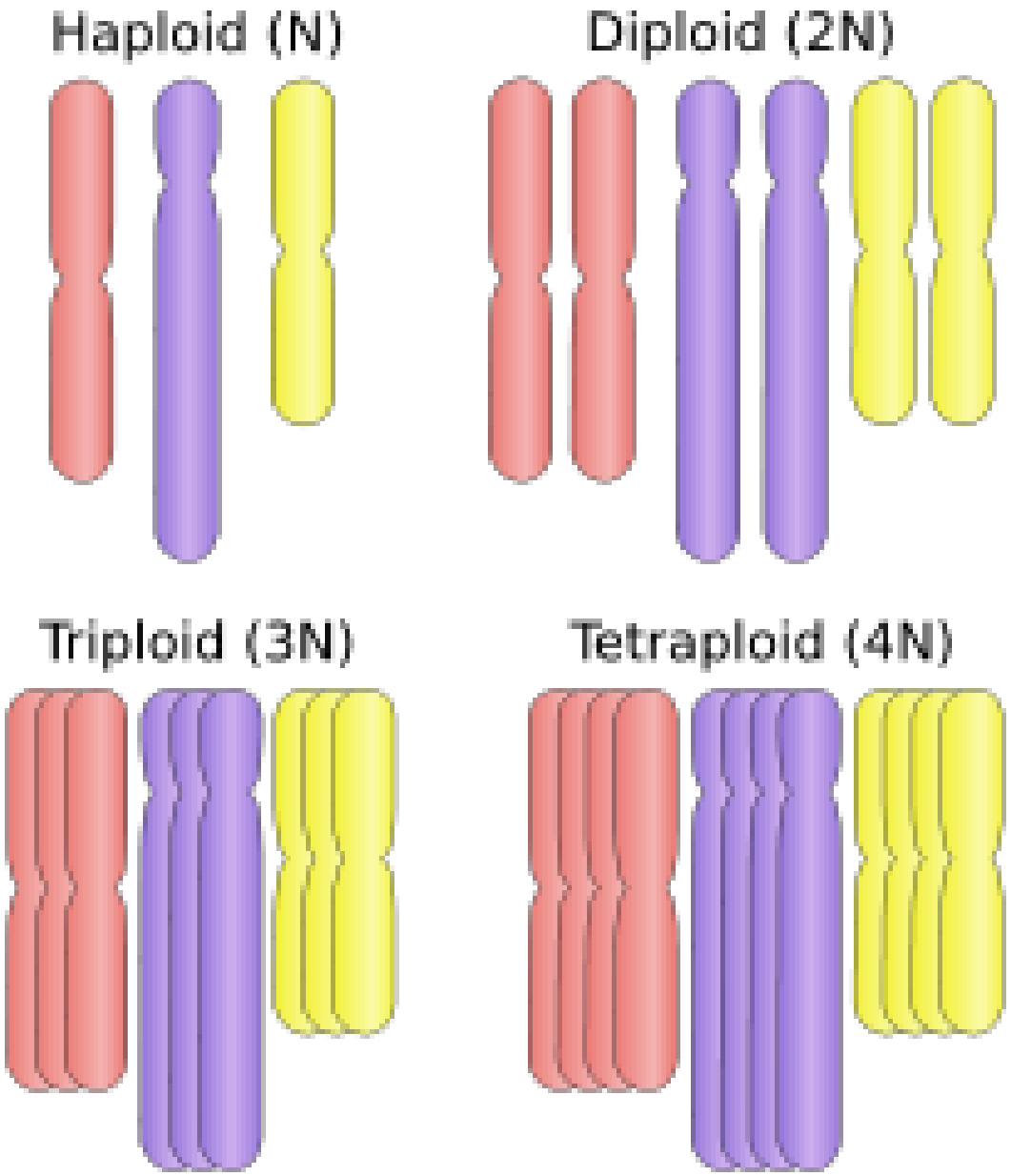
↓

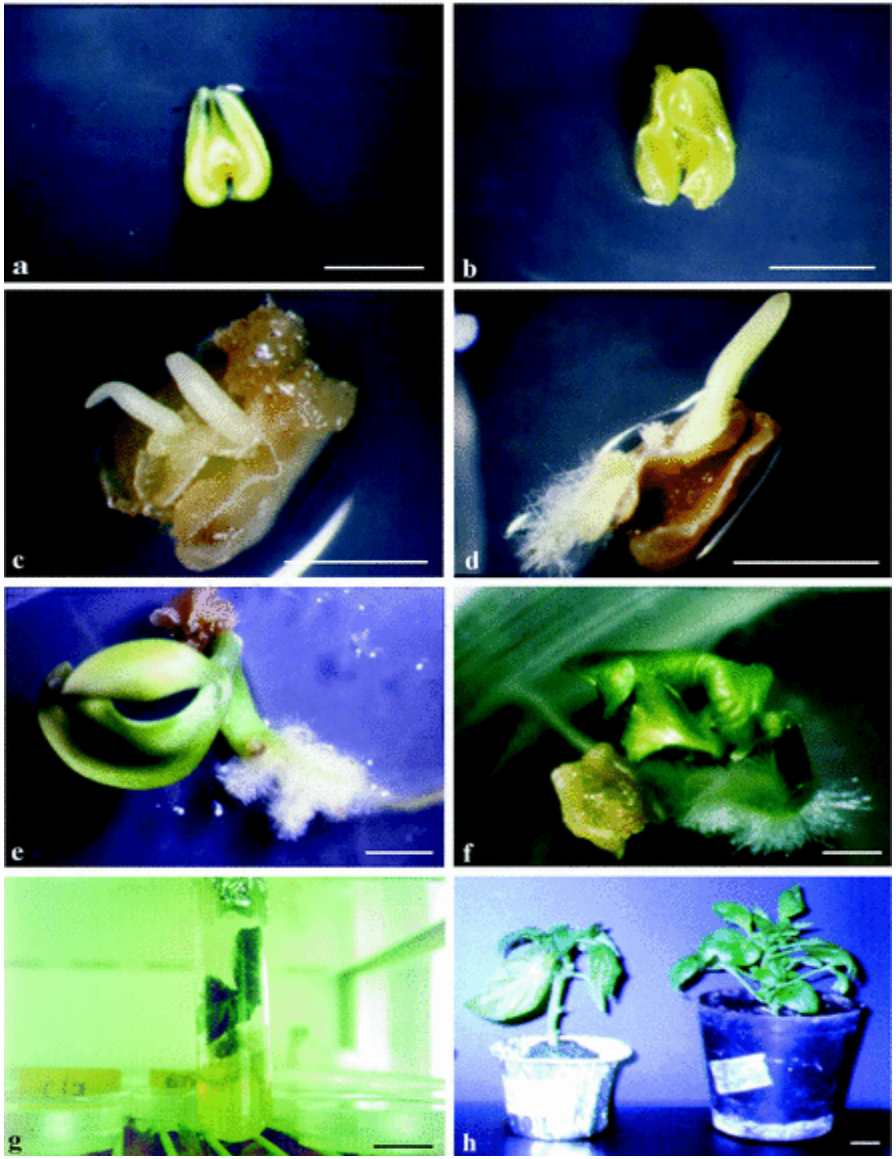
***T. aestivum* (AABBDD)**

The common bread wheat (*Triticum aestivum*) is an allohexaploid containing three distinct sets of chromosomes derived from three different diploid species of goat-grass (*Aegilops*) through a tetraploid intermediary (durum wheat).


Primitive egg cell
46 chromosomes total
Primitive sperm cell
46 chromosomes total
Normal meiosis
Error in meiosis
23 chromosomes
23 chromosomes
24 chromosomes
22 chromosomes
47 chromosomes
at fertilization
("Trisomy" results)
45 chromosomes
at fertilization
("Monosomy" results)
(2 pairs of chromosomes
drawn for simplicity, instead
of all 23 pairs)


Meiosis
Parent cell
DNA replicates
2 daughter cells
4 daughter cells

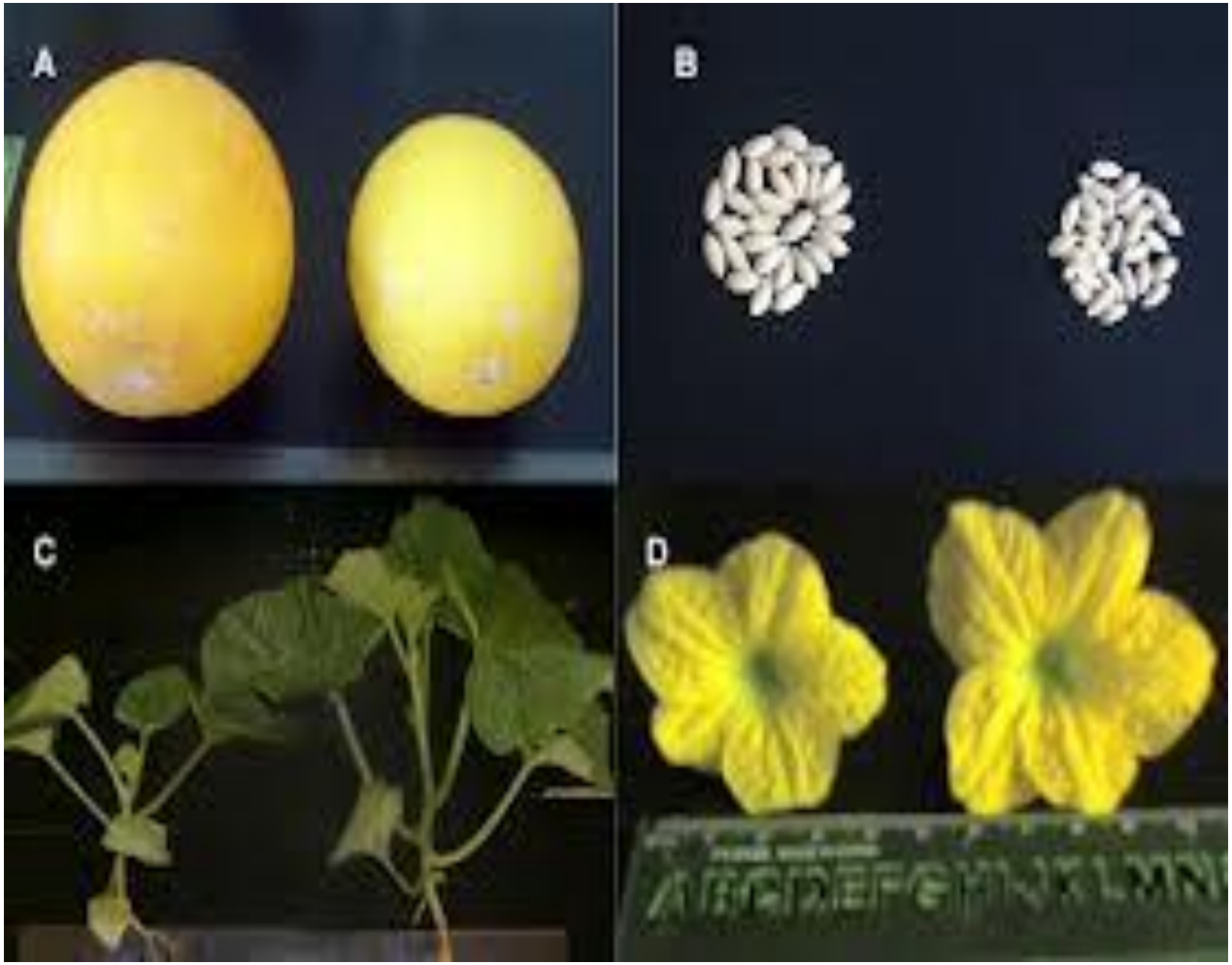

Haploid (N)
Diploid (2N)
Triploid (3N)
Tetraploid (4N)



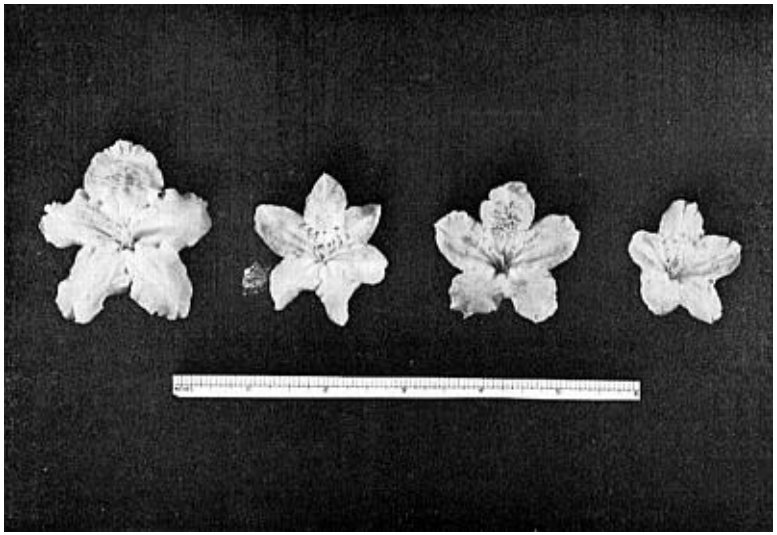
- After 6 days in culture, responsive anthers swelled and increased in size up to 1.5±0.2-fold (Figure 1b). Many other anthers became dark and lost turgescence. Later, 20 to 30 days, white elongated structures emerged from the responsive anthers (Figure 1c). These were identified as embryos, showing roots and shoots at later stages (Figure 1d). These embryos proceeded through the cotyledonal stage (Figure 1e), and finally towards green plantlets with leaves (Figures 1f and 1g). After 80 days, the regenerated plants developed a normal anatomy, but, in some cases, they were smaller with less leaves (Figure 1h, left-hand side) and grew less vigorously than control diploid plants (Figure 1h, right-hand side).

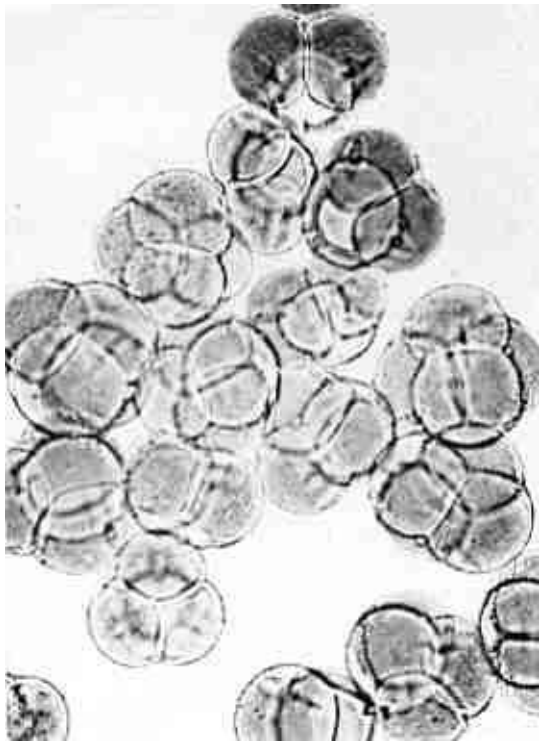
- In vitro induction of haploid wheat plants through anther culture and their subsequent transfer to pots. (A) Formation of callus in the cultured anthers. (B and C) Green and albino plantlets emerging from cultured anthers. (D) Plantlets in greenhouse. (E) Haploid chromosome number (*n* = 3X = 21). (G) Double haploid chromosome number (2*n* = 6X = 42). (H) Sterile plant (spike abnormal). (I) Fertile plants, normal spike and seeds obtained from the same plant.

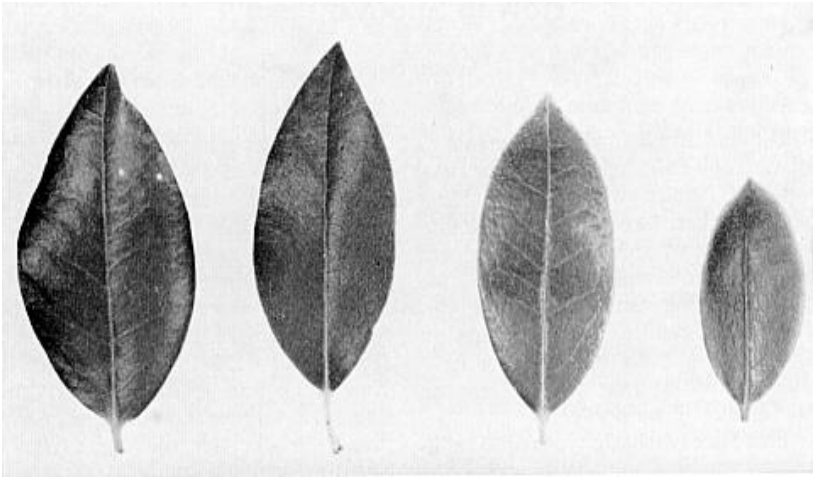
- ***a*. C.1 double haploid plant of 'Clemenules'**. *b*. G haploid plant of 'Clemenules'. *c*. Detail of blossom of the G haploid plant. *d*. Haploid and diploid flower of 'Clemenules'. *e*. B.1 aneuploid plant of 'Clemenules'.

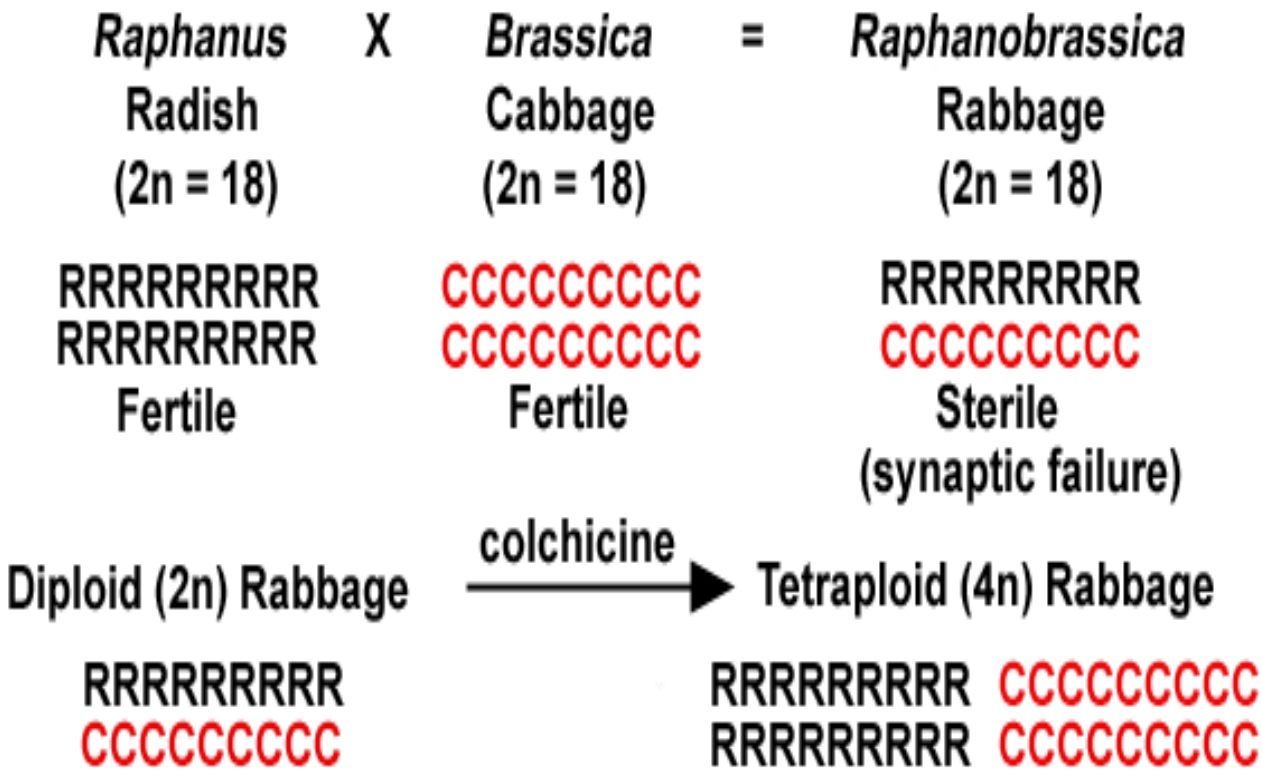


- Morphological characteristics of tetraploid and diploid muskmelon. (A) Fruit of tetraploid (left) and diploid (right), (B) seeds of tetraploid (left) and diploid (right), (C) 40-day-old plants of diploid (left) and tetraploid (right), (D) flowers of diploid (left) and tetraploid (right).





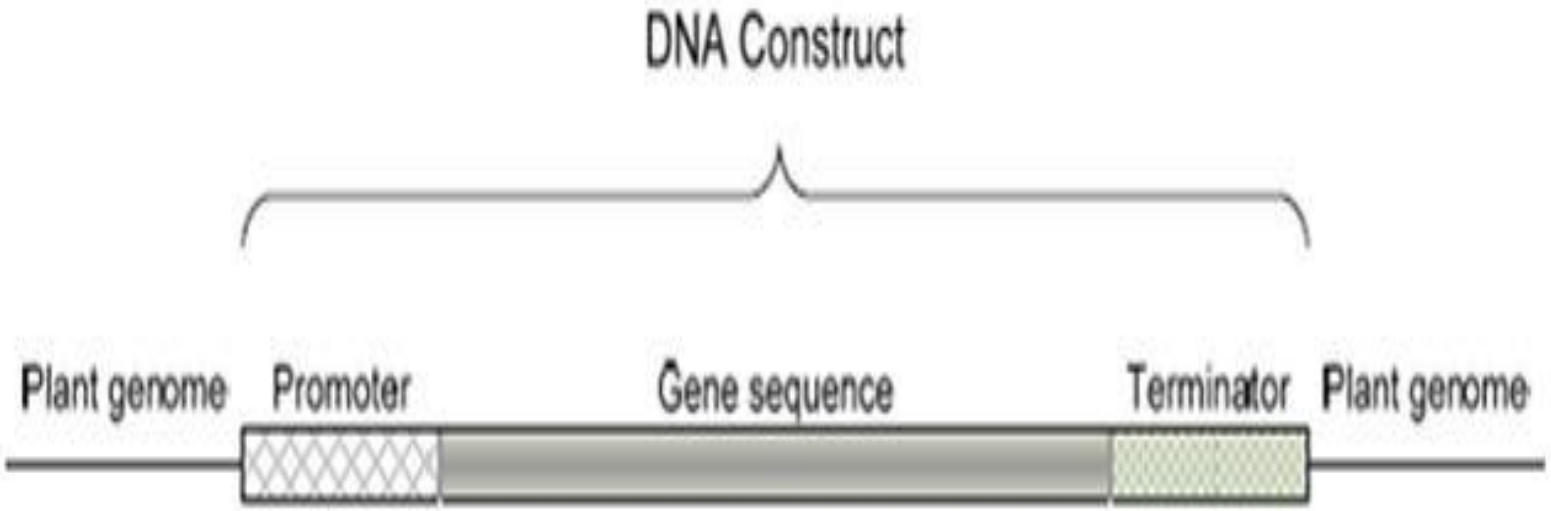


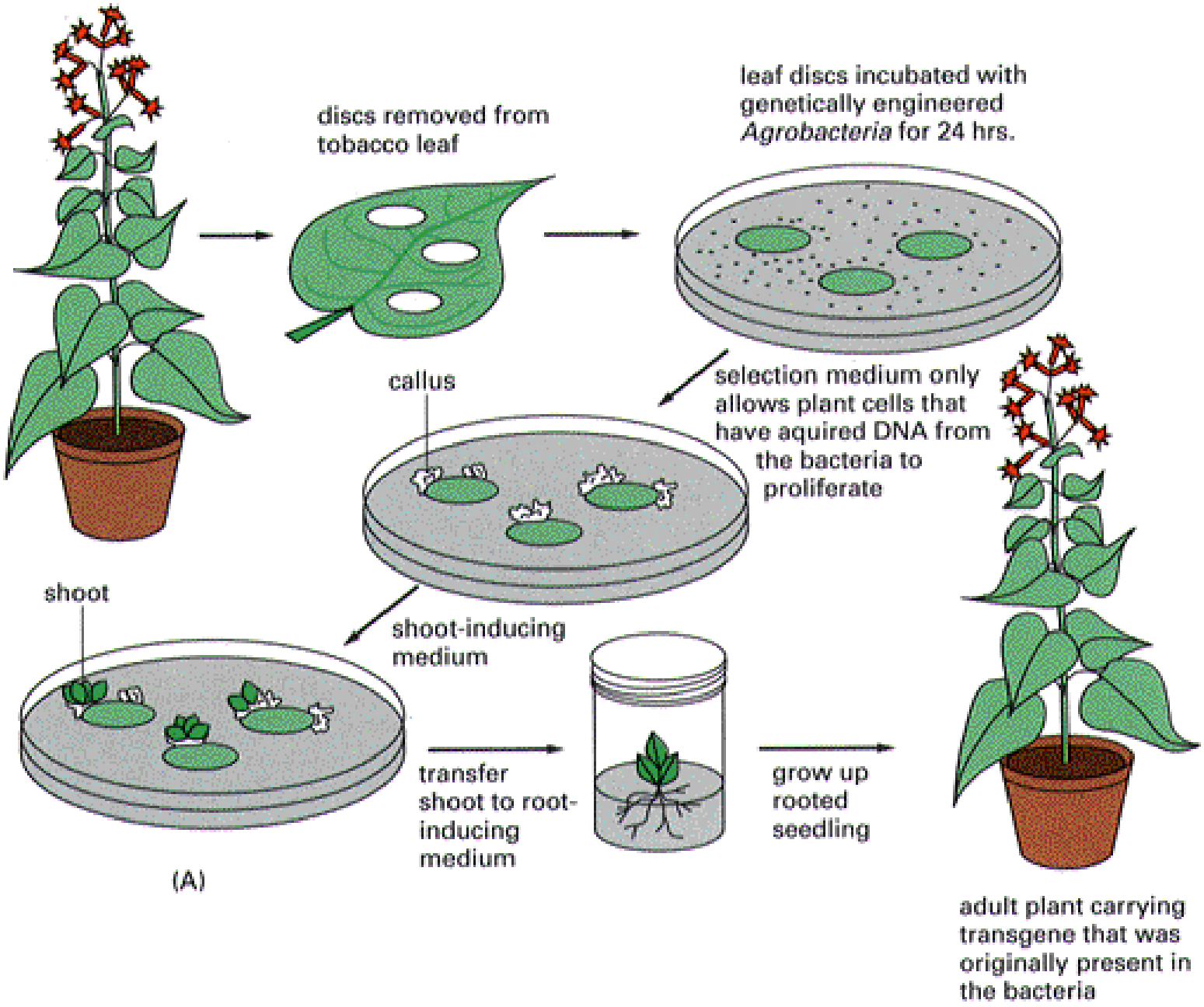


The Formation Of A Fertile Tetraploid Rabbage

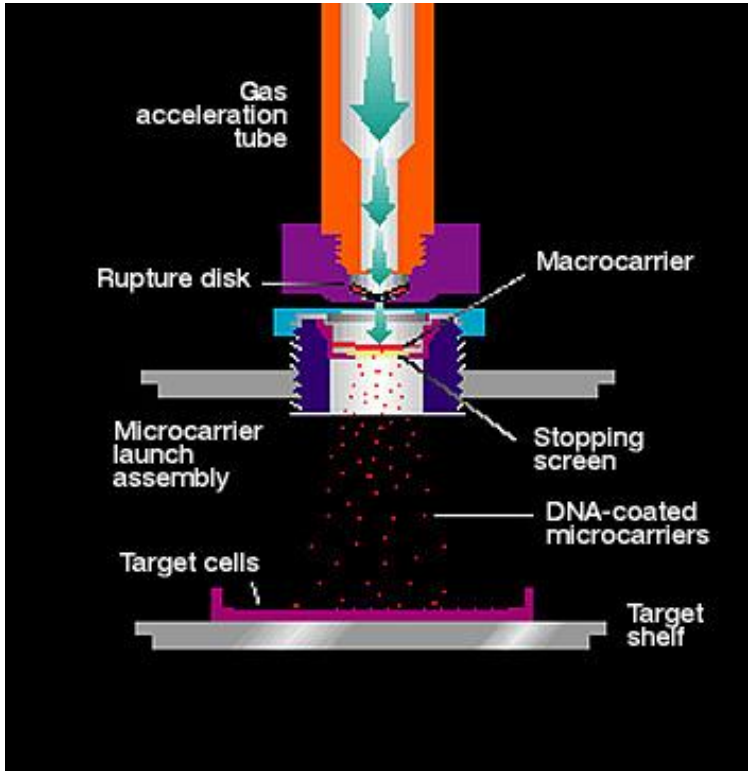
R = radish chromosome C = cabbage chromosome

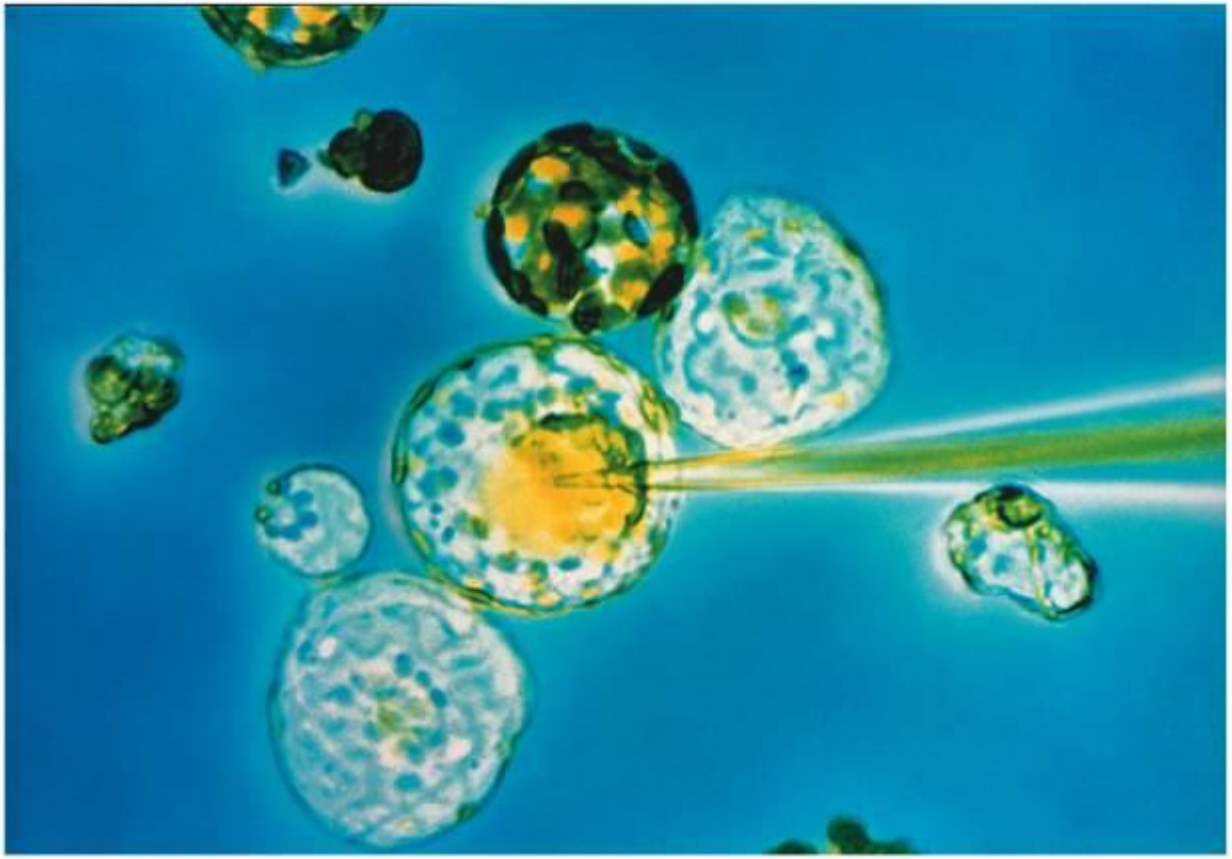
Gene Transfer

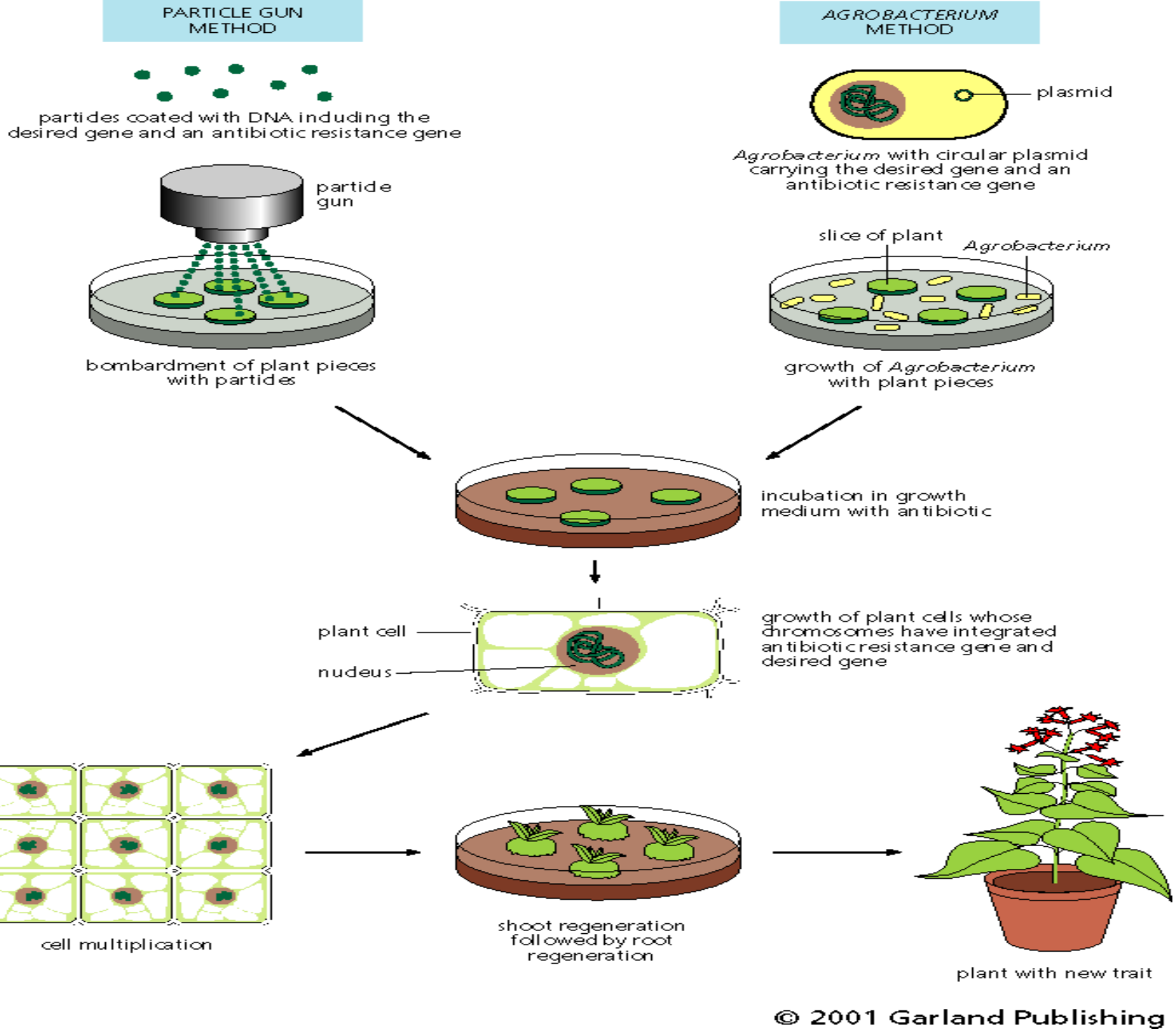

DNA Construct
Plant genome
Promoter
Gene sequence
Terminator
Plant genome



Gene Gun


Gas acceleration tube
Rupture disk
Macrocarrier
Microcarrier launch assembly
Stopping screen
DNA-coated microcarriers
Target cells
Target shelf




PARTICLE GUN METHOD
AGROBACTERIUM METHOD
partides coated with DNA induding the desired gene and an antibiotic resistance gene
partide gun
bombardment of plant pieces with partides
plasmid
Agrobacterium with circular plasmid carrying the desired gene and an antibiotic resistance gene
slice of plant
Agrobacterium
growth of Agrobacterium with plant pieces
incubation in growth medium with antibiotic
plant cell
nudeus
growth of plant cells whose chromosomes have integrated antibiotic resistance gene and desired gene
cell multiplication
shoot regeneration followed by root regeneration
plant with new trait
© 2001 Garland Publishing

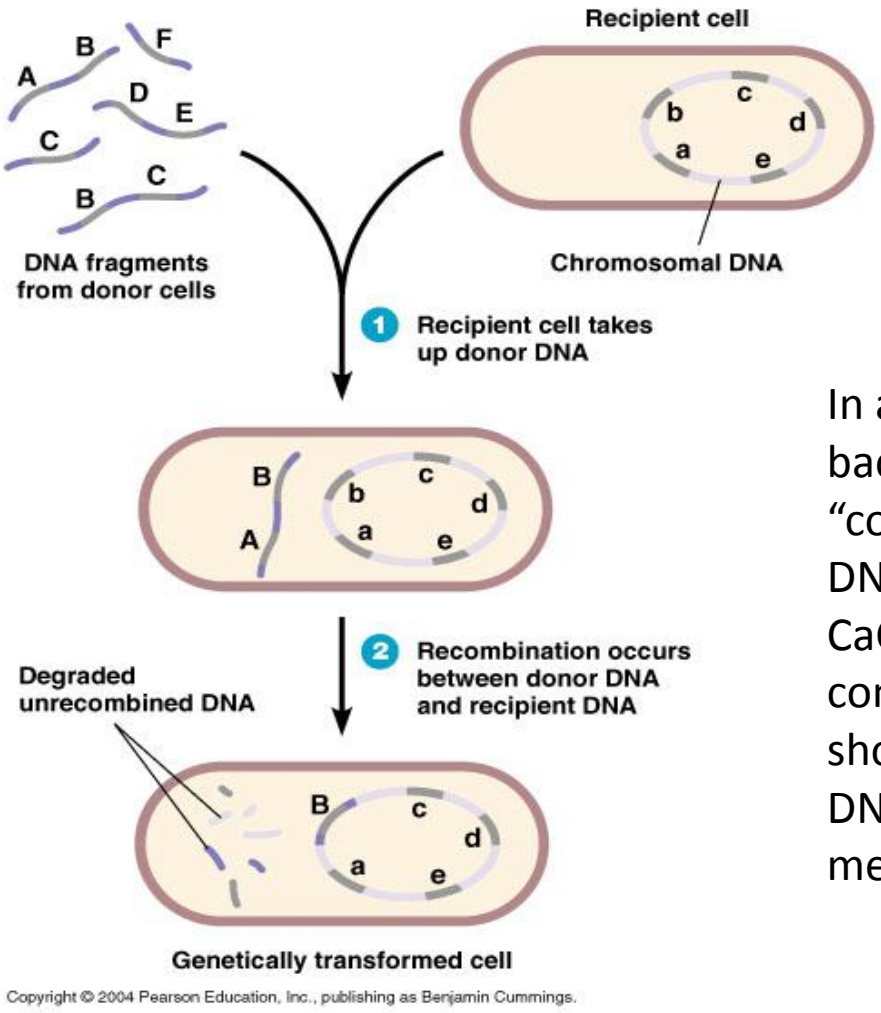
Process

1. Restriction enzymes cut out gene that is to be transferred
2. The same restriction enzyme cuts into the plasmid
3. Because it is the same restriction enzyme, the same complementary 'sticky ends' in the plasmid

Process

4. DNA ligase joins the sticky ends, fixing the gene into the E.coli plasmid
5. The recombinant plasmid is inserted into the host cell.
6. It now expresses the new gene.

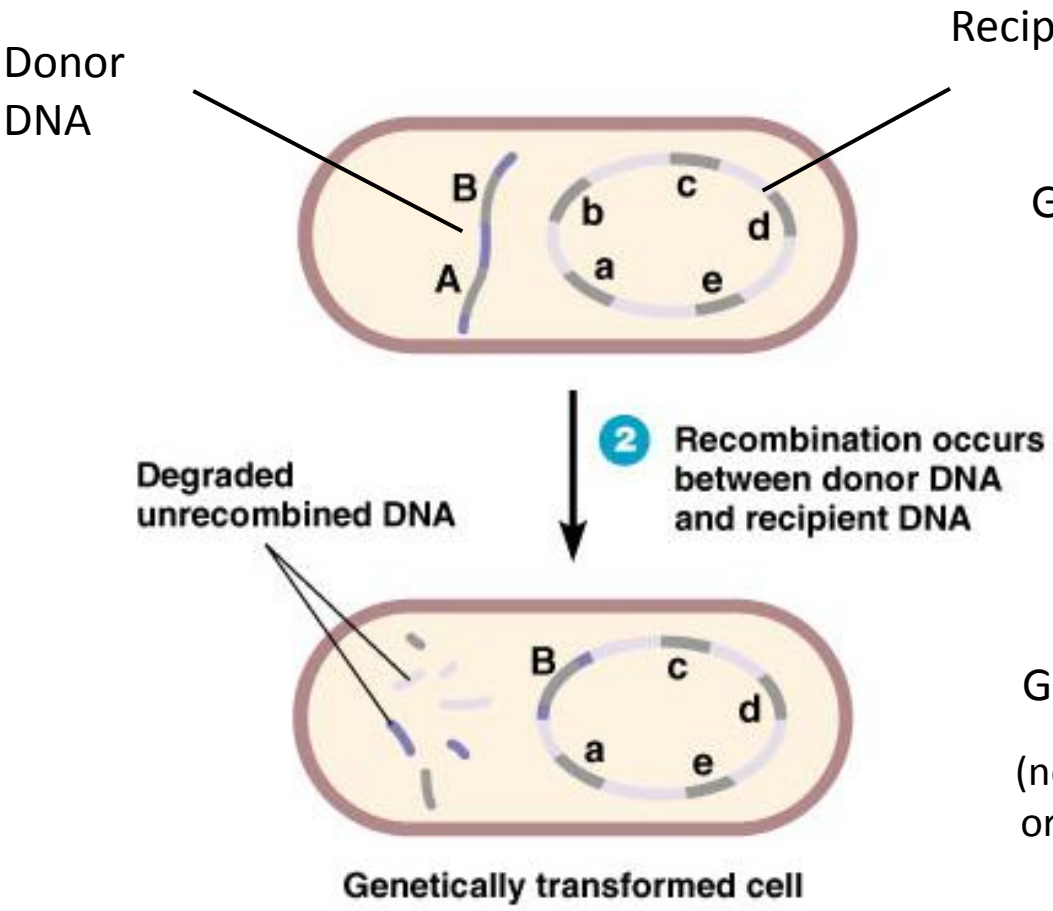
Transformation: Absorption of Free DNA



In a lab setting, many bacterial species are not "competent" to take up donor DNA. They are incubated in $CaCl_2$ to make them competent and then heat shocked so they will suck up DNA fragments from the medium.

Figure 8.24

Recombination Produces New Genotypes



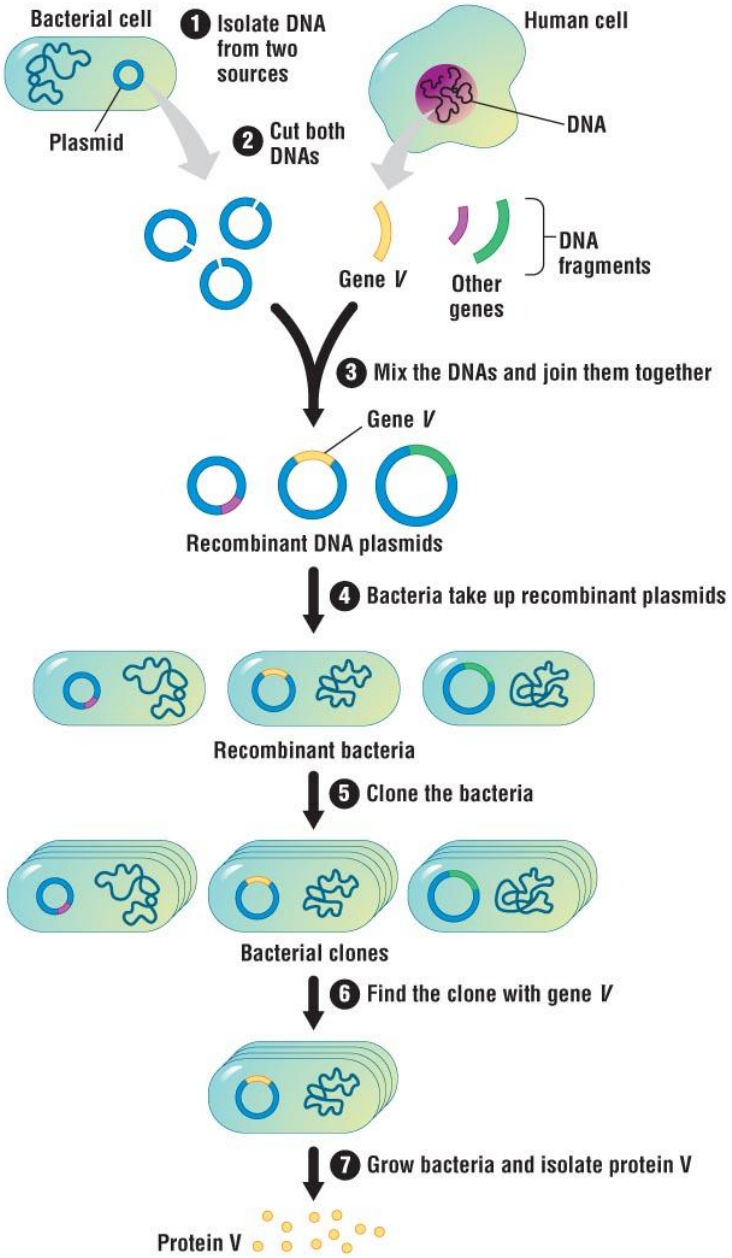
Genotype: abcde

Genotype: a**B**cde

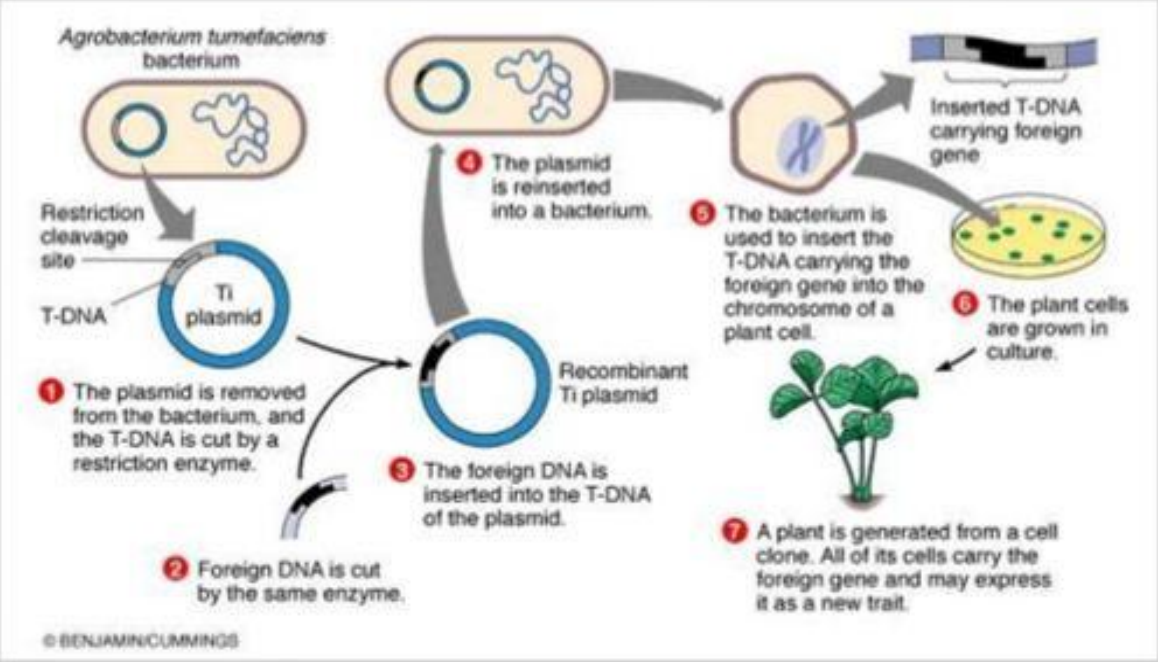
(now has a new **phenotype** or ability, like being able to use a sugar it could not before)

Figure 8.25

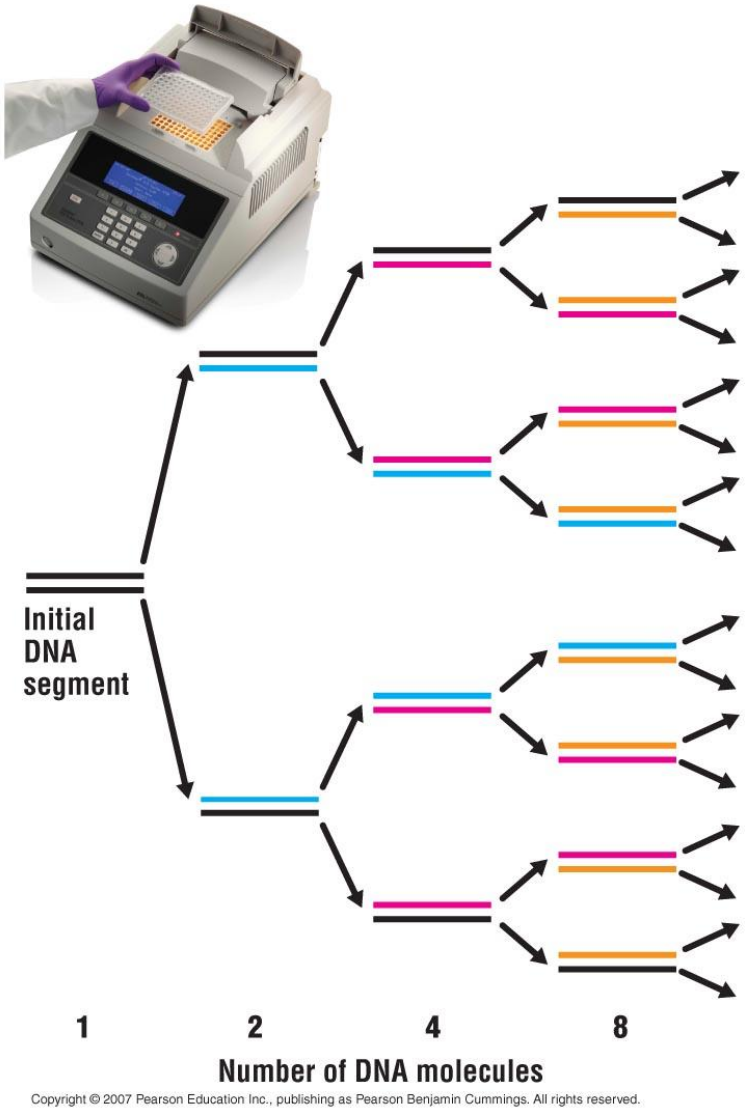
Genetic Engineering Involves Insertion of a Gene on a Plasmid



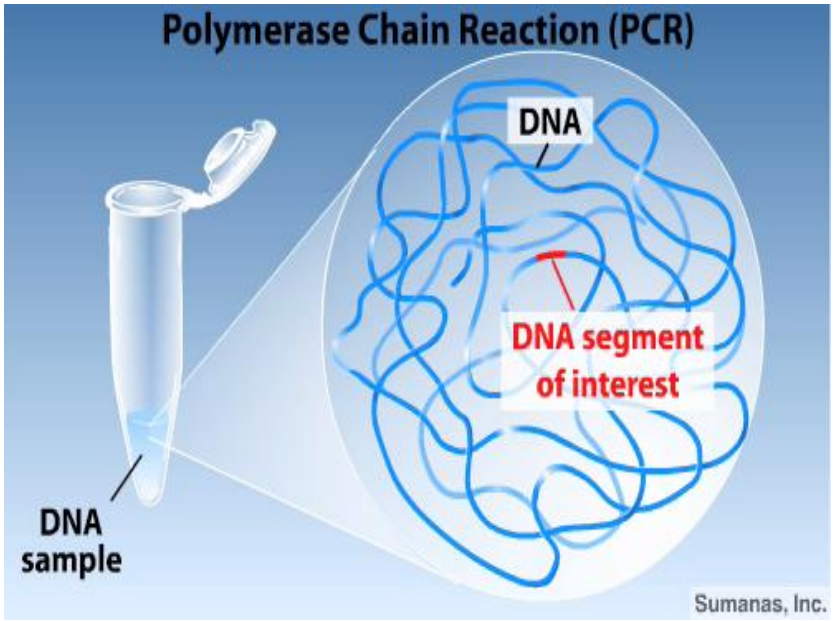
An example


Agrobacterium tumefaciens bacterium
Restriction cleavage site
T-DNA
Ti plasmid
1 The plasmid is removed from the bacterium, and the T-DNA is cut by a restriction enzyme.
2 Foreign DNA is cut by the same enzyme.
3 The foreign DNA is inserted into the T-DNA of the plasmid.
Recombinant Ti plasmid
4 The plasmid is reinserted into a bacterium.
5 The bacterium is used to insert the T-DNA carrying the foreign gene into the chromosome of a plant cell.
Inserted T-DNA carrying foreign gene
6 The plant cells are grown in culture.
7 A plant is generated from a cell clone. All of its cells carry the foreign gene and may express it as a new trait.
© BENJAMIN/CUMMINGS

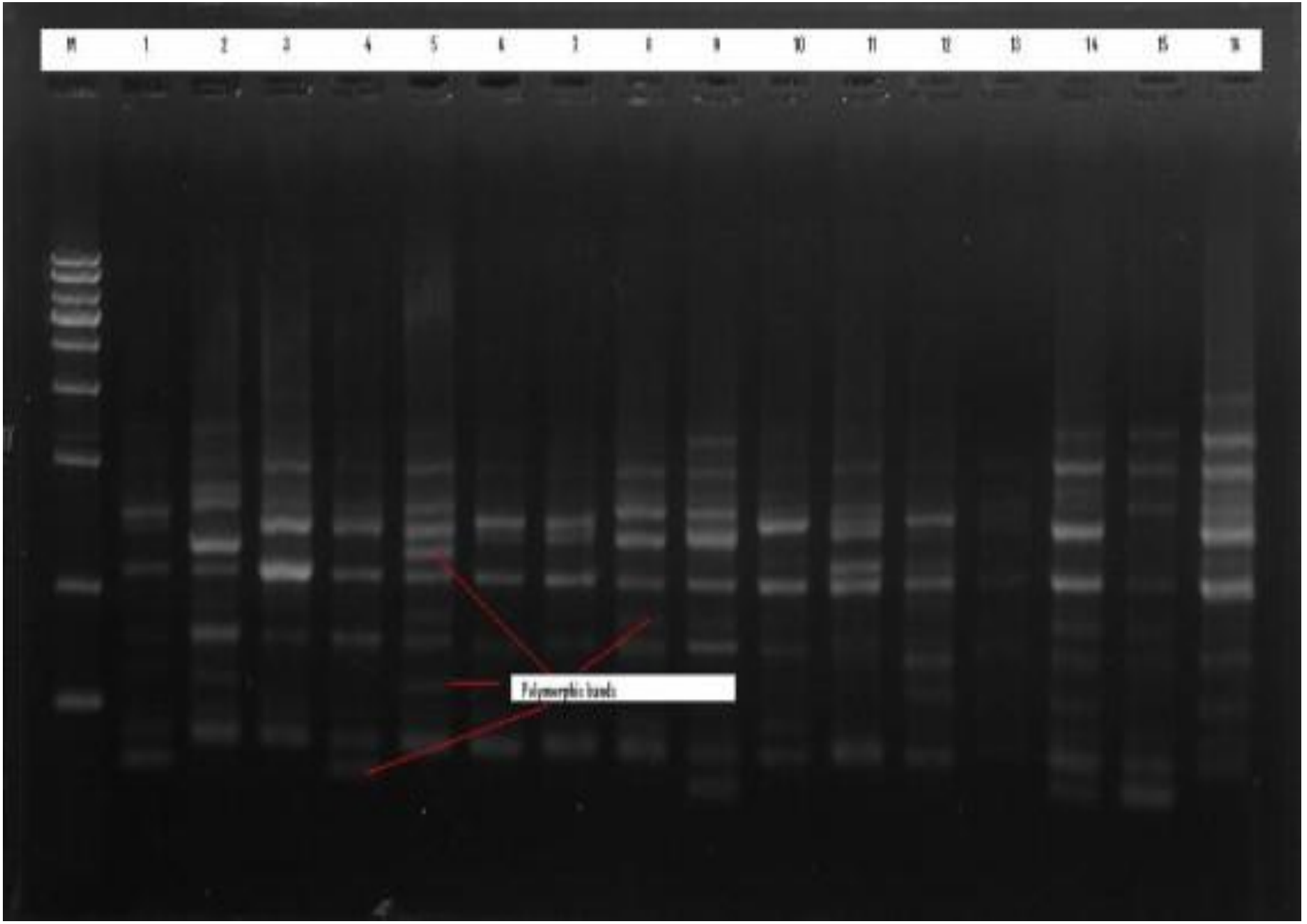
Polymerase Chain Reaction: DNA Replication in a Test Tube

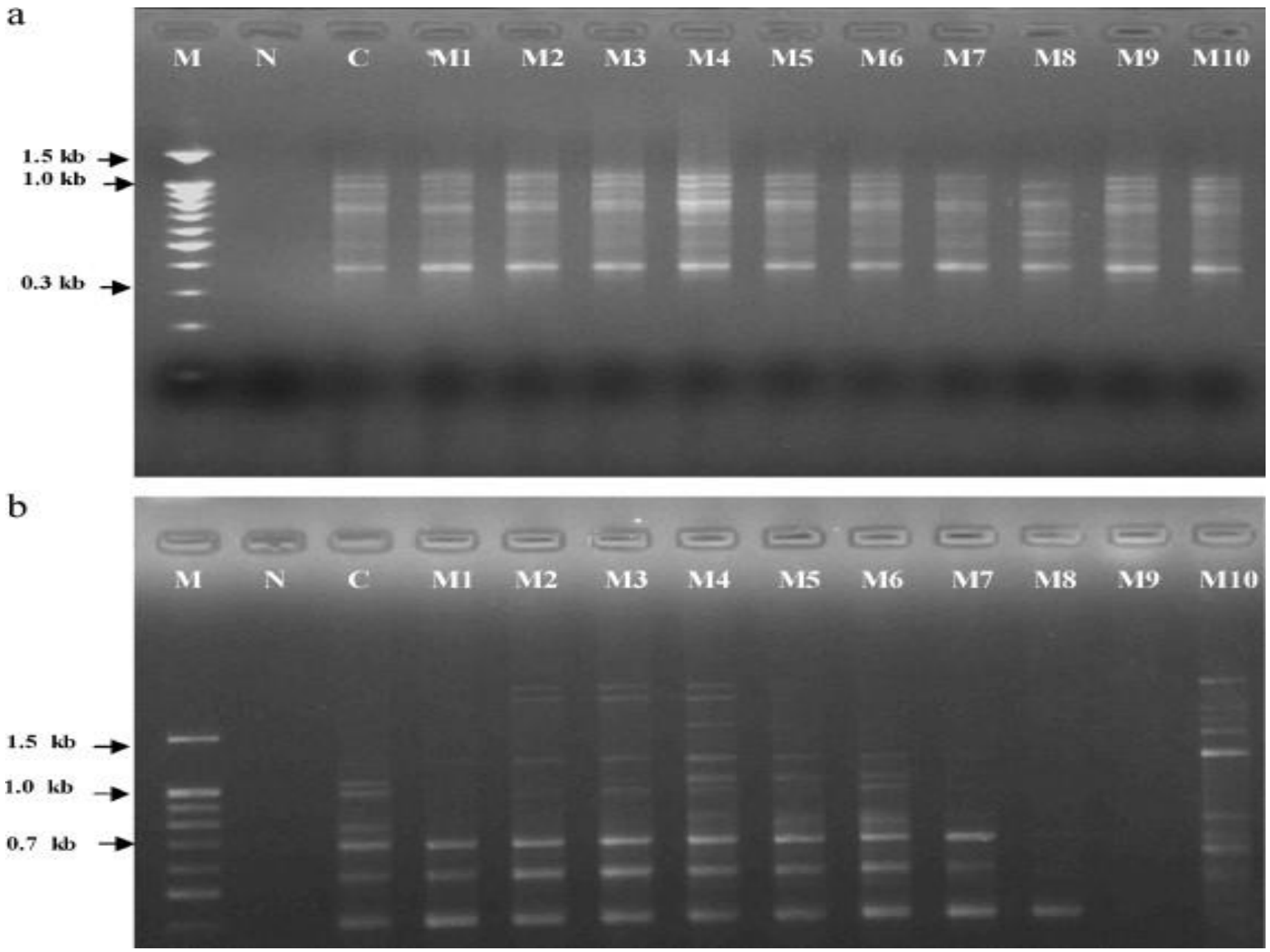


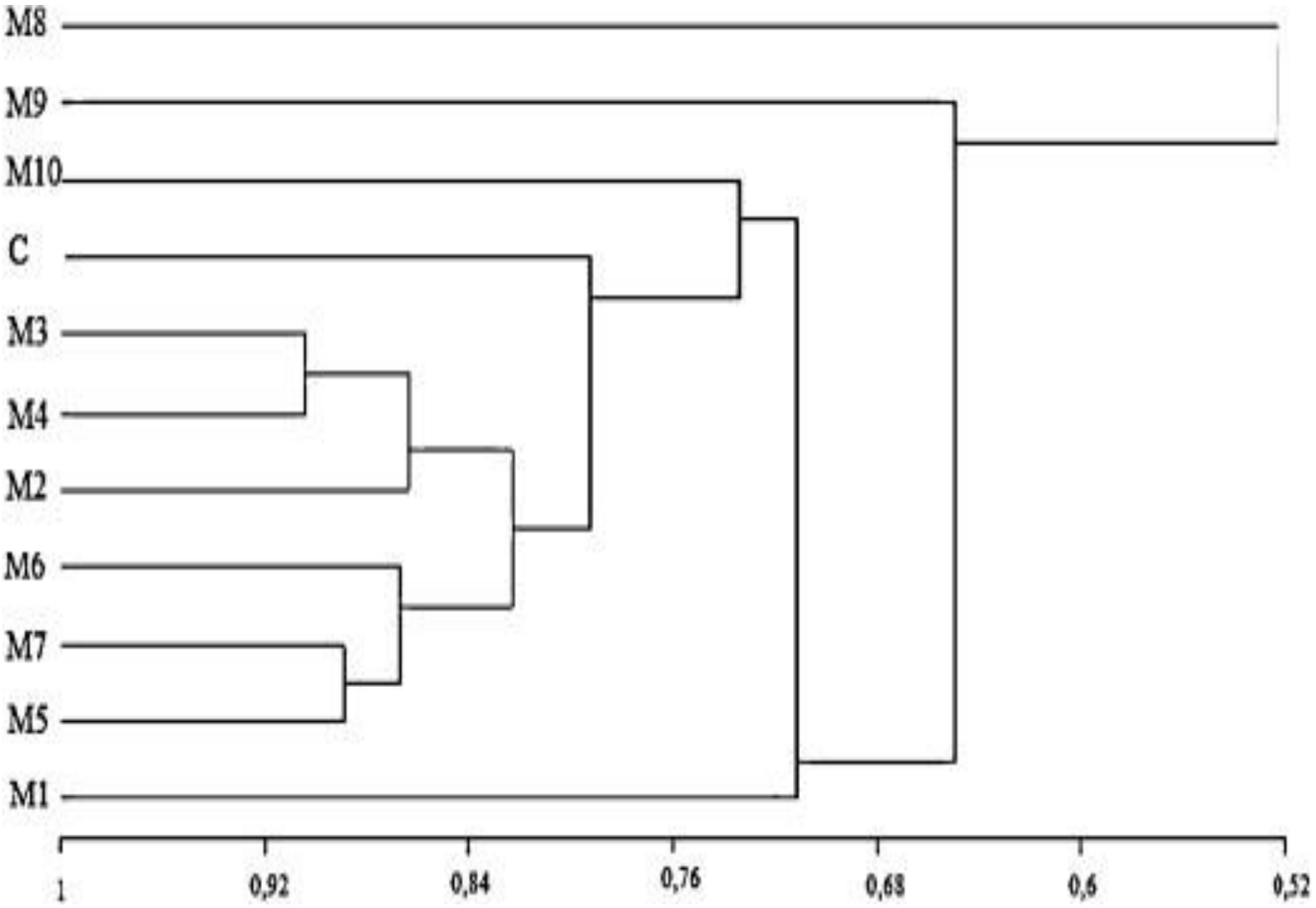
Exponential Increase in the Number of DNA Molecules each Cycle


Polymerase Chain Reaction (PCR)
DNA
DNA segment of interest
DNA sample
Sumanas, Inc.


Polymerase Chain Reaction


M
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Polymorphic bands


a
M
N
C
M1
M2
M3
M4
M5
M6
M7
M8
M9
M10
1.5 kb
1.0 kb
0.3 kb
b
M
N
C
M1
M2
M3
M4
M5
M6
M7
M8
M9
M10
1.5 kb
1.0 kb
0.7 kb


M8
M9
M10
C
M3
M4
M2
M6
M7
M5
M1
1
0,92
0,84
0,76
0,68
0,6
0,52