Natural and induced mutations secured by clonal propagation: impact and implications

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PLANT BREEDING

- Plant breeding has been defined as the art and science of changing the traits of plants in order to produce desired characteristics

- This can be achieved in various ways from simply selecting plants with desirable characteristics for propagation, to more complex molecular techniques.

HOW CAN WE IMPROVE CROPS?

Conventional breeding

1. Breeding/Hybridization followed by selection
2. **Identification and selection of natural mutations**
3. **Radiation/Chemical Mutagenesis**
4. Cloning – Grafting, budding, tissue culturing

New breeding Technologies

1. Site-Directed Nucleases (SDN) (including ZFN-1/2/3 and CRISPR systems);
2. Oligonucleotide Directed Mutagenesis (ODM);
3. Cisgenesis;
4. RNA-dependent DNA methylation (RdDM);
5. Grafting (non-GM scion on GM rootstock);
6. Reverse breeding;
7. Agro-infiltration

MUTATIONS & MUTATION BREEDING

Mutation (De Vries (1901)

- A sudden, heritable change in the genetic material, which was not due to segregation or recombination.

Mutation breeding

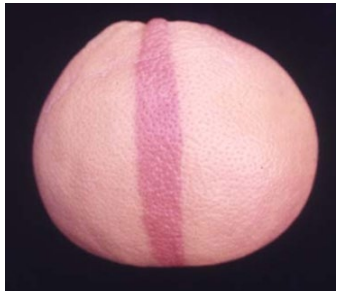
- Mutation breeding refers to the method of using artificial mutagenesis to induce a change that would have occurred naturally to obtain new biological cultivars, mainly through chemical or radiation mutagenesis.

ROLE OF MUTATION BREEDING

- Many crops = natural bud mutations
- Supplementary to conventional breeding
- Induced mutation = breeding method for crops that never form seeds

Advantages:

- alter (improve) single characteristic
- rest of genotype unchanged
- improved traits are added,
- time required shorter than with hybridization
- benefit even higher if trait can be secured through vegetative propagation to be a commercial clone
- recessive traits



Constraints:

- Chimera formation = main problem
- Selection only from mV2 (second generation) onward

IMPORTANCE OF MUTATION BREEDING

1. Linear increase in interest from 1977 to 2018 - trend still ongoing
2. In 2018 there were 3222 mutant varieties released worldwide in over 200 crop species, as compared to 571 mutant varieties in 84 crop species in 1977.
3. These include 20 different fruit species having more than 50 cultivars
4. Mutation derived cultivars have contributed billions of dollars to the economies of many countries.
5. Main beneficiaries are developing countries, but first world countries also benefited.
6. Impact was on modified oil, protein and starch quality, enhanced uptake of specific metals, deeper rooting system, and resistance to drought, diseases and salinity as a major component of the environmentally sustainable agriculture.
7. Mutation in fruit breeding contributed mostly towards mitigating conventional breeding constraints and enhancing quality aspects
8. **ARCCIT9 – +R1.7 million trees planted in 11 years.**

MUTATION BREEDING AND PLANT VARIETY PROTECTION

Plant Variety Protection (PVP) is a tool to **foster innovation** towards **long-term solutions** in agriculture, horticulture and forestry through a **lengthy** and **expensive** process requiring **skills** and accumulated **knowledge** that are applied in a **scientific approach.**

PVP and mutations = EDV

ESSENTIALLY DERIVED: THE LAW

(b) For the purposes of subparagraph (a)(i), a variety shall be deemed to be **essentially derived from another variety** ("the initial variety") when:

i. it is **predominantly derived from the initial variety**, or from a variety that is itself predominantly derived from the initial variety, while retaining the expression of the **essential characteristics** that result from the genotype or combination of genotypes of the initial variety,

ii. it is **clearly distinguishable from the initial variety** and

iii. except for the differences which result from the act of derivation, it **conforms to the initial variety** in the expression of the **essential characteristics** that result from the genotype or combination of genotypes of the initial variety.

REGISTRATION AND DISTRIBUTION OF PLANT MATERIAL

- Plant Breeders' Right obtained based on
  - **New, Distinct, Uniform, Stable**
- Fruit producers can still receive a product that is not uniform or stable
  - Breeding procedures
  - Source of bud wood
  - Crop processes (Virus cleansing etc.)
  - Overzealous commercialisation
- Questions
  - % variation allowed for changed attribute?
  - Back mutation (reversion)
  - Re-testing of original material after 5 years?
  - When is it an EDV and who should benefit from an EDV?

OBJECTIVES THAT CAN BE ACHIEVED WITH MUTATION BREEDING

- Seedlessness/ low seediness
- Improved internal, external colour pigmentation
- Improved quality
- Change in ripening time
- Disease resistance

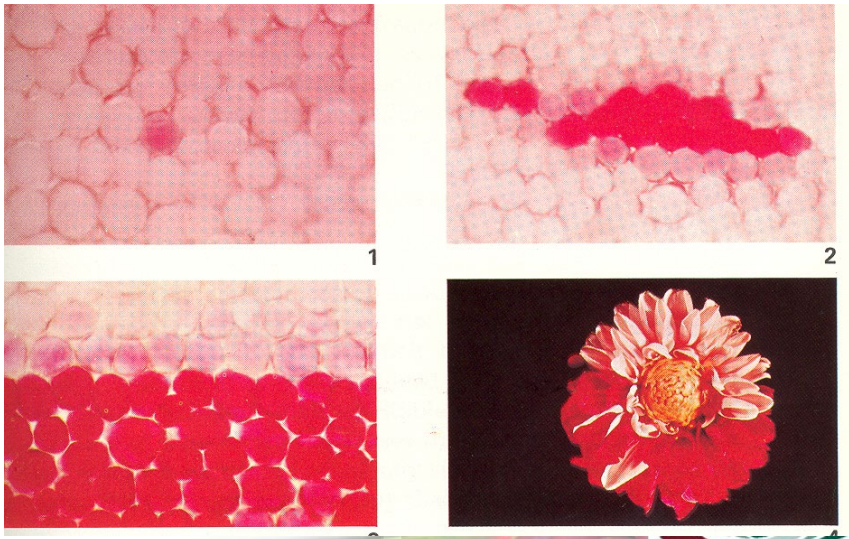
THREE LINKED STRATEGIES IN TREE CROP BREEDING

- Conventional
- Mutation
- Biotechnology

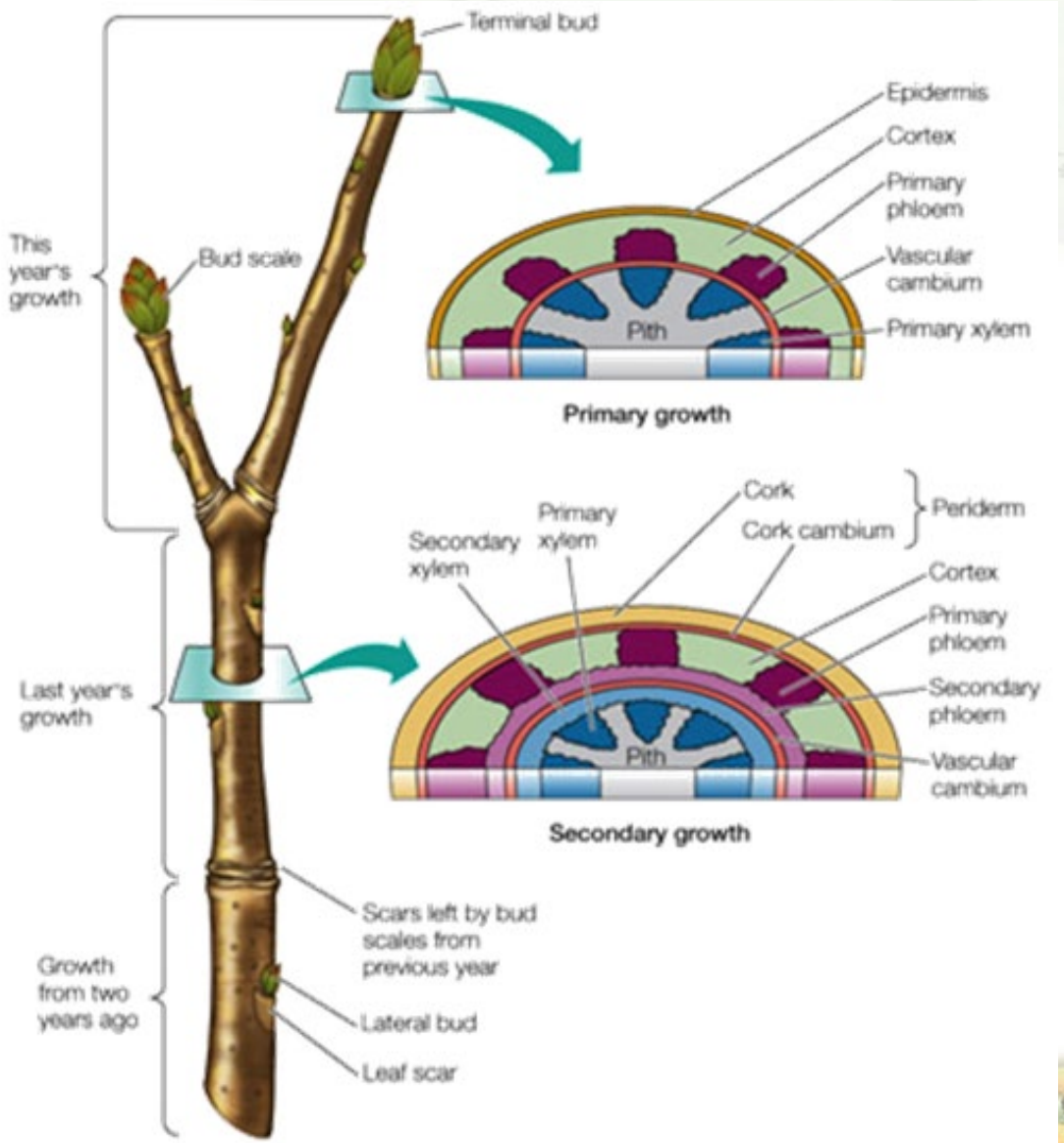
New improved cultivars

MUTAGEN TREATMENT AND HANDLING OF TREATED MATERIAL

- The success of a breeding project depends on the recognition of the desired genotypes and their recovery
- A mutation = one-cell event in a number of cell layers such as the epidermis and sub-epidermis with a number of meristematic cells in each layer
- Chimera formation in most cases results in mericlinal chimeras, subsequently developing in periclinal branches, shoots, tubes etc.



DEFINITION OF CHIMERA IN PLANTS



A chimera is a plant with two or more genetically dissimilar tissues growing side by side.

In general terms it is called “sports”

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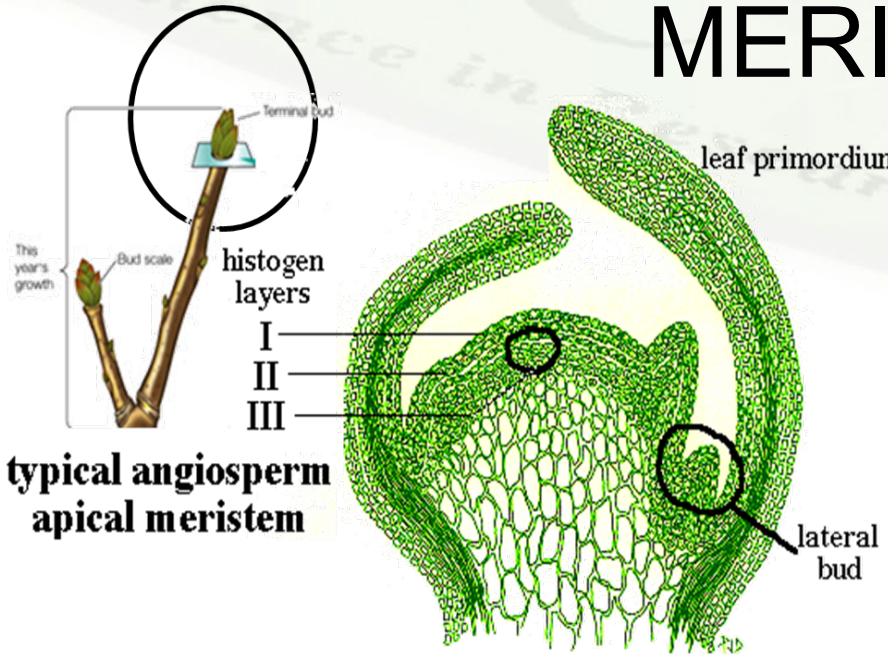
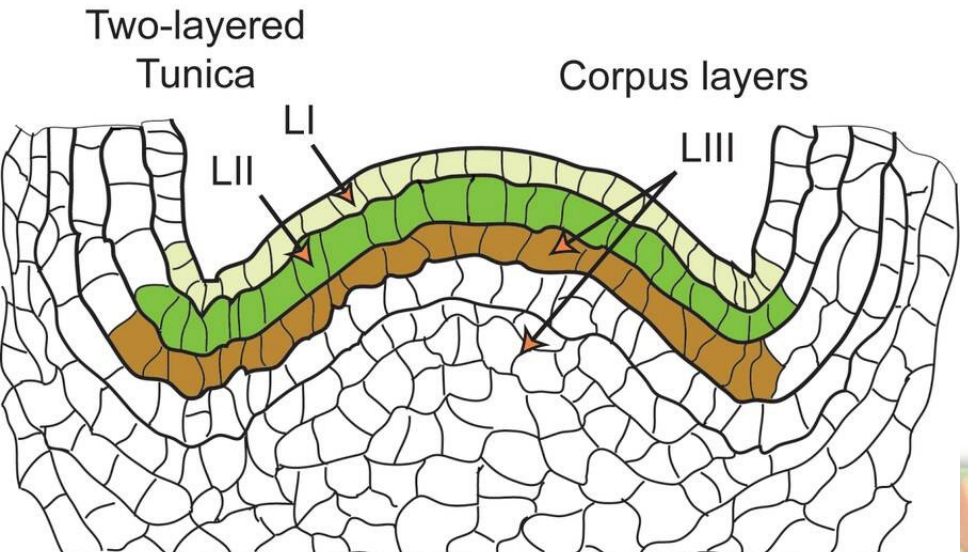
THE **TUNICA-CORPUS** THEORY OF MERISTEM



Higher plants have layered meristems that originate from a few cells in the center of the shoot apical meristem.

A plant's apical meristem or shoot tip is made up of relatively independent layers.



This is known as the **tunica-corpus** theory of meristem organization, where cell layers or tunica cover the body or corpus of the stem.

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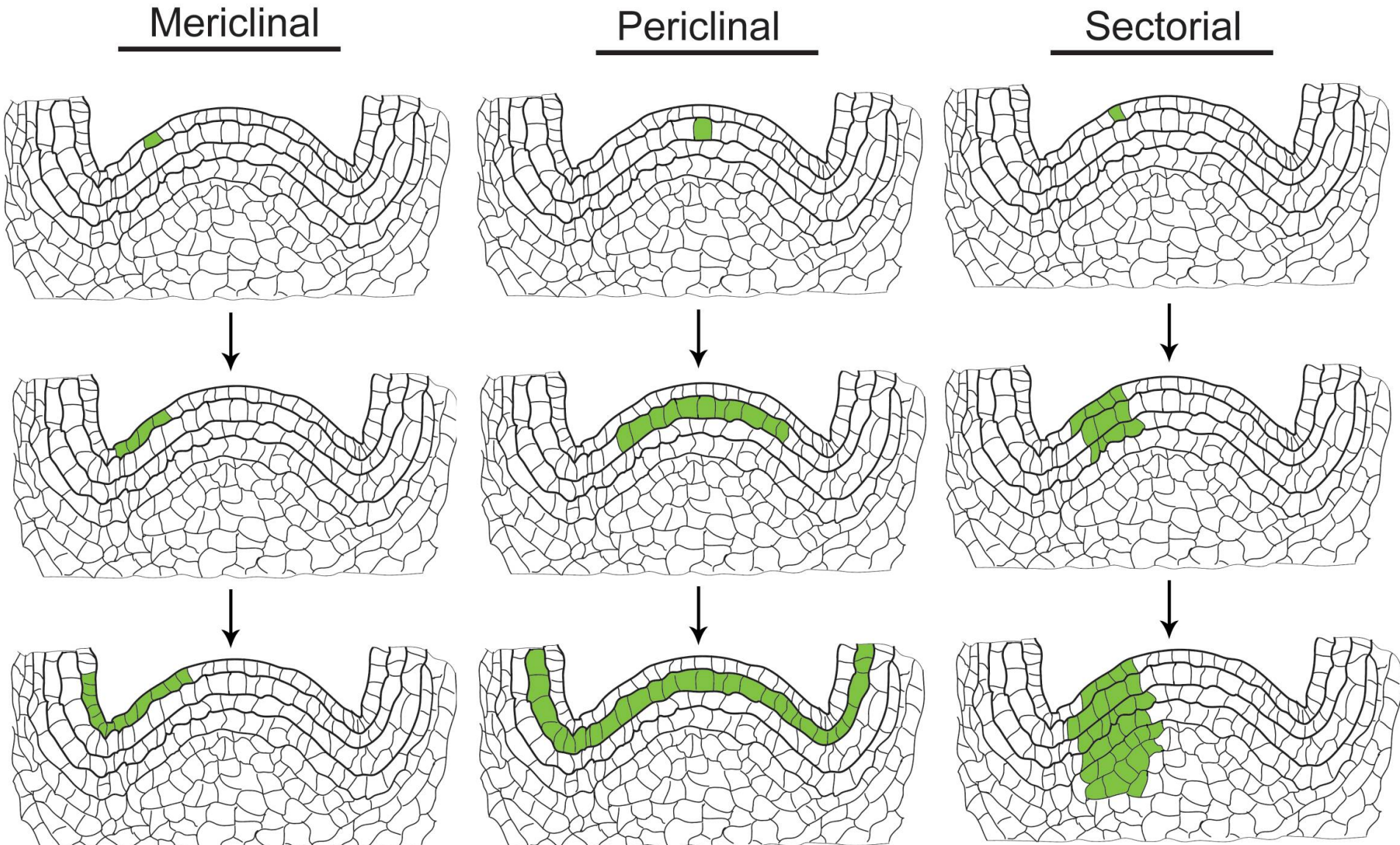
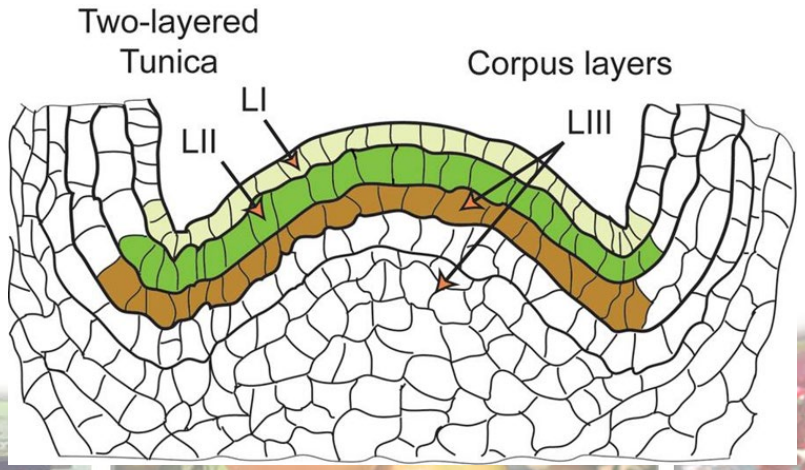
PATTERNS OF GENETIC CHIMERAS WITHIN CLONES



SHOOT CHARACTERISTICS

- **All organs in plants develop from the apex.**
- **In flowering plants, the vegetative developing part consists of the L1 (dermatogen), L2 (sub-dermatogen) and L3 (corpus)**
  - **From the L1 comes the epidermis**
  - **From the L2 comes the mesophyll and gametes**
  - **From the L3 comes the vascular bundle, the roots etc.**



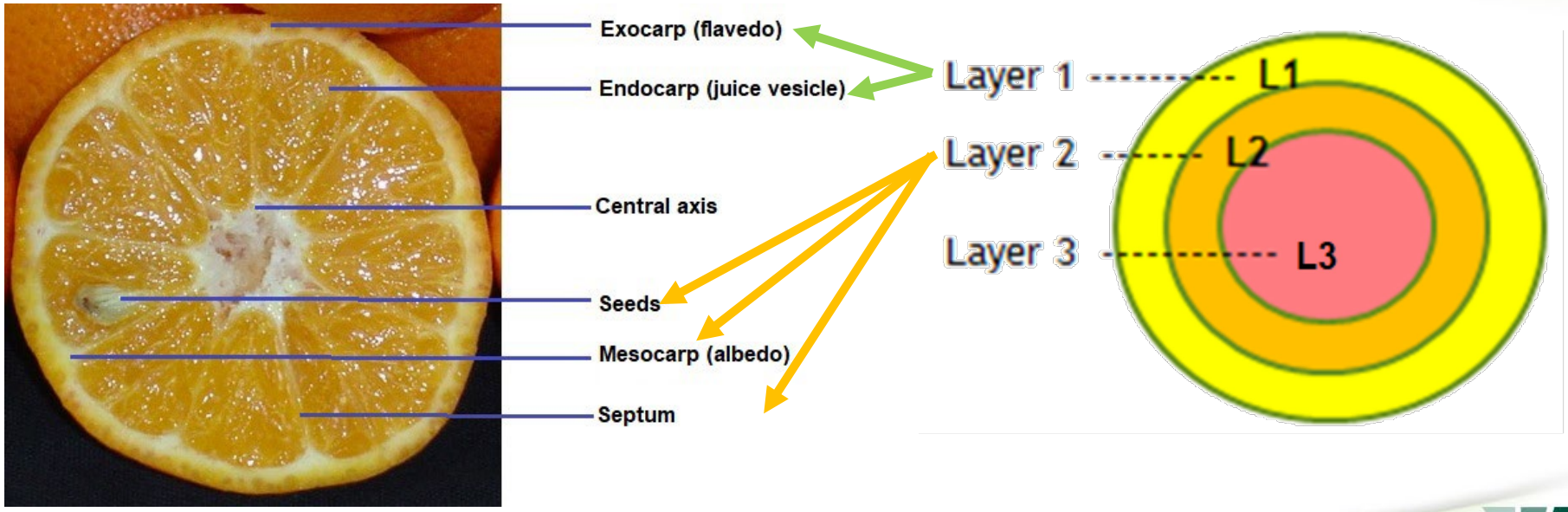
FRUIT CHARACTERISTICS

In citrus:

Layer 1 produces the juice sacs and the epidermis of the pericarp (rind)

Layer 2 produces seeds, segment walls, hypoderm and the mesocarp (albedo) of the epidermis

Layer 3 produces the vascular bundles



IRRADIATED/MUTATED BUDS

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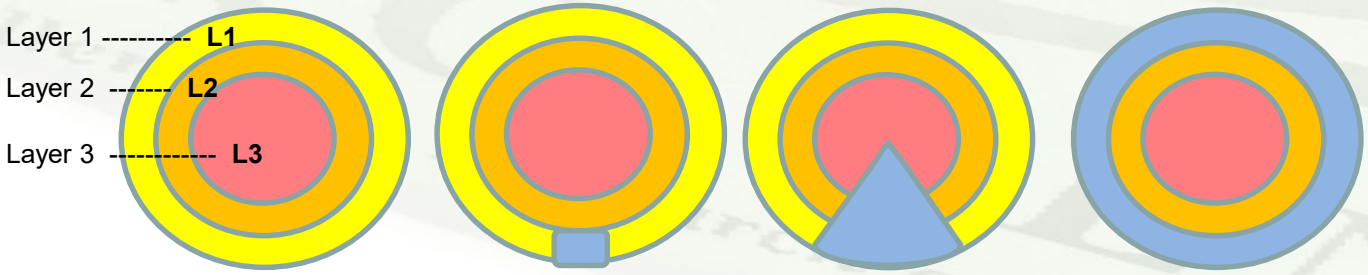
MUTATION SCENARIOS IN BUDWOOD: Cross section of shoots



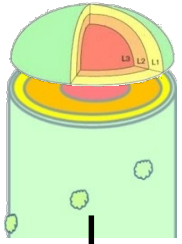
Non- mutated shoot

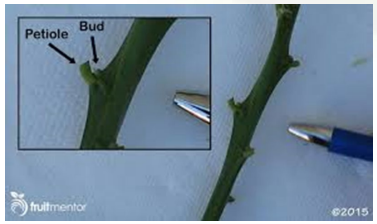
Mericlinal chimera
(L1 partly mutated)

Sectorial chimera

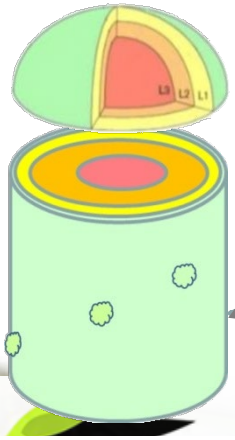
Periclinal chimera
(L1 mutated, stable)

Irradiate

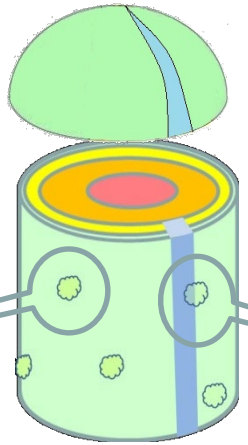




Irradiated Budwood for propagation

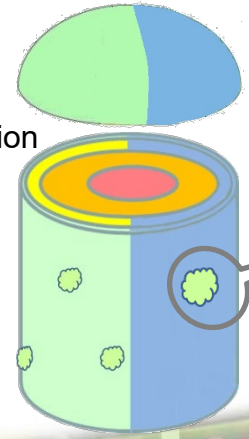


Vegetative propagation
(Auxiliary bud out of mutated area)

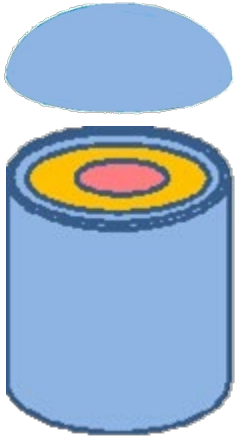


Mericlinal Chimera in budwood
(Mutated partly in L-1)

Vegetative propagation
(Auxiliary bud partly in mutated area)



Vegetative propagation



Periclinal mV2 Chimera
(Fully mutated L-1 part)

Non-mutated mV1 plant
(Loss of mutated part)

Mericlinal mV1 Chimera
(Enlarged mutated L-1 part)

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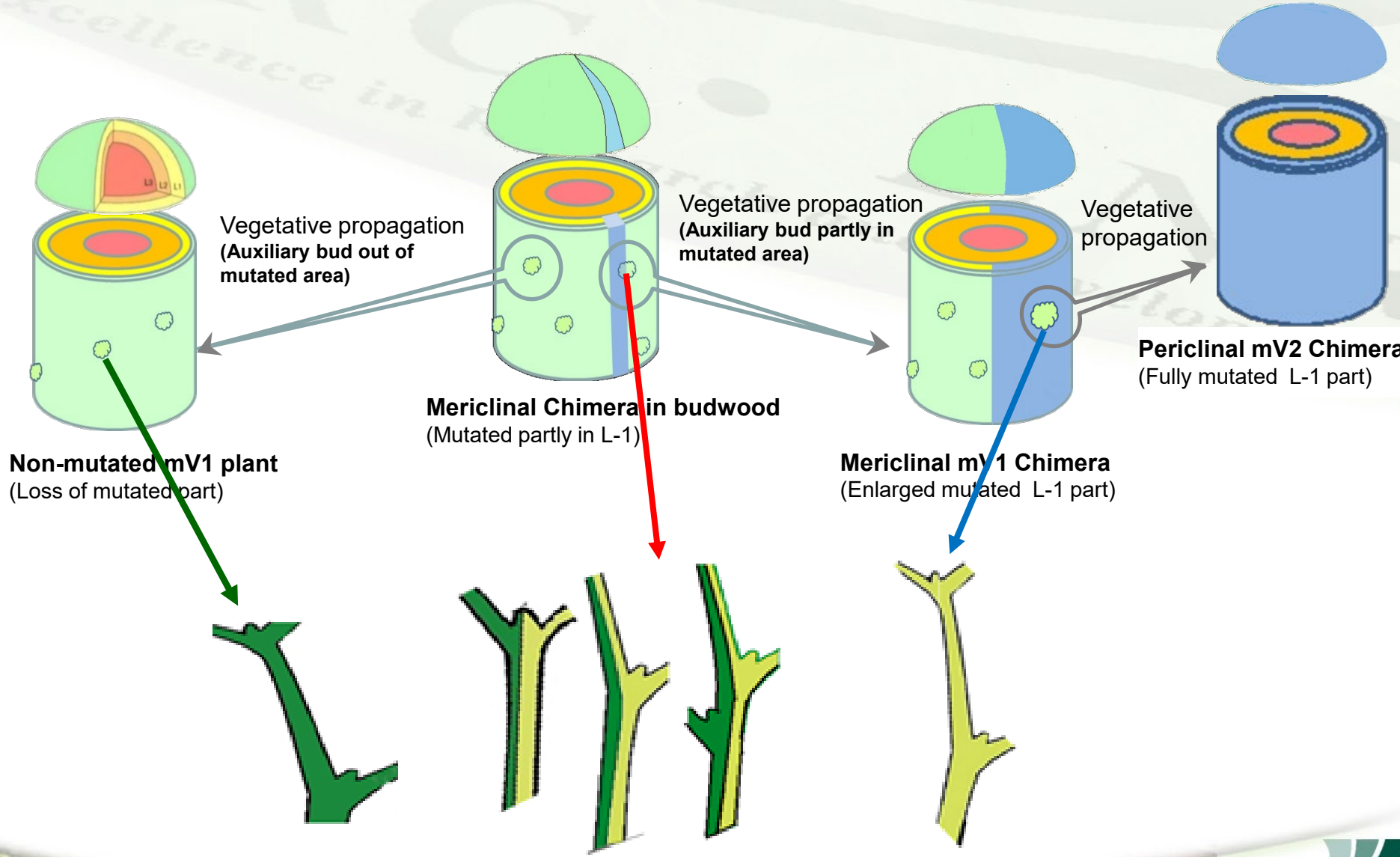
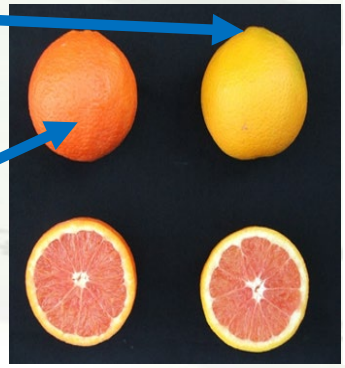
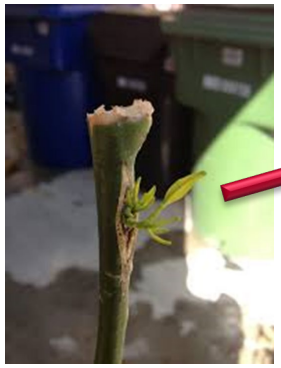
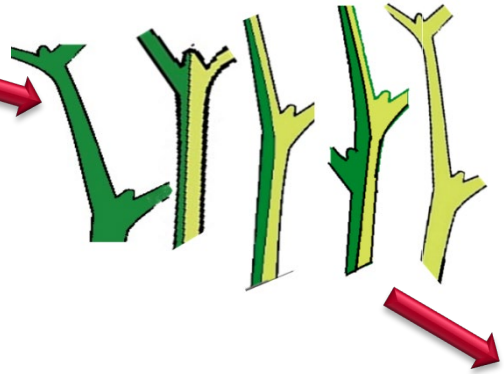
MUTATION SCENARIOS: Buds per budwood section









IMPLICATION OF CHIMERIC BUDWOOD BASED ON ORANGES

- Mutations secured by clonal propagation can be detrimental to the commercial sector in the absence of systematic phenotypic analysis

- Multiple cycles of phenotypic clonal testing is essential to test the stability of a mutation whether it is an induced or natural occurring mutation

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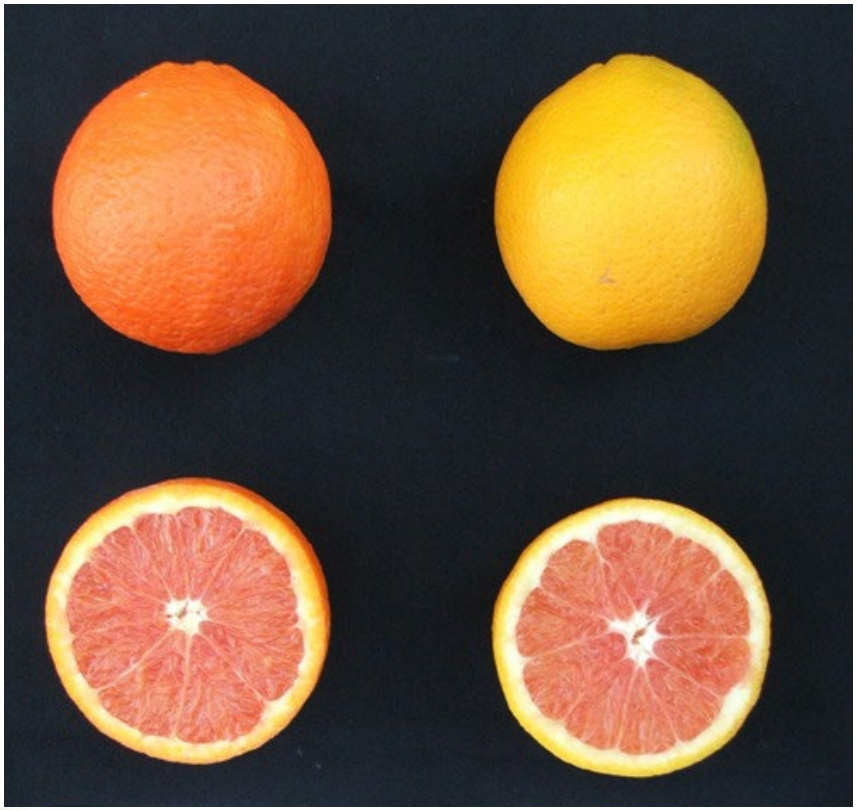
STABLE MUTATIONS

Navels:

Dark skinned Cara Cara



Eureka! Seedless Lemon™ (ESL)

Ensuring uniform and stable cultivars from mutation breeding	
PHASES	**PROCEDURE**
Mutation phase	Induce mutations
M1 (M1V1)	Bud the mutagens treated buds onto a rootstock and plant in the field
M2 (M1V2) (Discovery of natural mutations)	Evaluate the (M1V1) plants for mutations Select mutants and take the bud directly behind the fruit that display the mutation and bud again to a rootstock and plant in the field.
M3-M8	Continuing selection, genetic confirmation, multiplication and stabilisation of field performance of mutant lines. During this phase, should there be branches that still display mutated and non-mutated fruit then the procedure of M2 is repeated on a branch where most or all the fruit is mutated.
Next generation	During this generation the material that are now deemed pure is multiplied for comparative analysis of mutant selections during different years and in different locations.
Next Phase	Application for Plant breeder's rights and subsequent release follows as soon as soon as stability and performance has been verified.

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CONCLUSION

- Mutation breeding is important
- PBR's for EDV's are important
- UPOV depends on integrity of the applicant
  - Proof and submission of work done?
- Uniformity and stability of the trait
  - Re-evaluation after 5 years?
- What was deemed essential in the original cultivar and what % thereof is retained ?
- Change must be on an important attribute (commercial)
- Breeding vs Discovery
- Magnitude of the inputs in development of selection

Thank You