

INTRODUCTION

- The goal of vegetative propagation is to select a single source plant of superior characteristics and to reproduce populations of progeny plants with identical genotypes that are its direct descendants.
 - This biological process is described as **cloning**, and the resulting population of plants as **clones**.






Figure 16-1 (a) Monterey Pine (*Pinus radiata*) 0-1-year-old rooted layer cuttings after 1 year in stoolbed, and (b) 11-year-old, uniform stand of clonal *P. radiata* for timber production in New Zealand (see circled individual in photo for scale reference).

Hartmann and Kester's Plant Propagation Principles and Practices 8e
Hudson Hartmann, Dale Kester, Fred Davies and Robert Geneva

PEARSON

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Clone

- The vegetative progeny of a single genotype.
 - individual seedling
 - a mutant branch
 - a single plant of a clonal population
 - a recombinant DNA segment



Propagule

- Any plant part used as the starting point of a propagation process.

Clonal Selection

- The process of selecting an individual plant or plant part to create a clone.



Advantages

- Genetic improvement and selections
- Uniformity of populations
- Control of phases of plant development
- Combine more than one genotype into a single plant.



Disadvantages

- Monoculture
- Slow reproduction rate
- Potential for genetic variation
- Potential for systemic pathogens



Traditional Breeding Seedling Selection



Origins of Clones as Cultivars

Seedling selection

- Grapes ‘Cabernet Sauvignon’ 2K
- Pear ‘Bartlett’ 1770
- Apple ‘Delicious’ 1870
- Nuts
- Roses
- Chrysanthemum

Mutations and “Bud-Sports”

Mutation – permanent genetic change involving some part of the DNA molecule.

1. rearrangement of bases in the DNA structure - **Point mutations**.
2. rearrangement of parts of the chromosome - **Deletions, duplications, translocations and inversions**.

Mutations and “Bud-Sports”

3. Addition or subtraction of individual chromosomes - **Aneuploidy**.
4. Multiplication of entire sets of chromosomes - **Polyploidy**.

Most mutations are deleterious but occasionally a bud-sport appears that has some horticultural advantage.

Induced Mutations

- The rate of mutation may be increased.
 - Mutagenic agents
 - X-rays, gamma rays, neutrons, and chemicals
 - Mutation breeding
 - Development of new clones with the use of mutagenic agents



Biotechnology

- Cell and tissue culture technologies
- Recombinant DNA technology
 - A gene from one organism is inserted into the genome of another individual.
 - Roundup Ready cotton



Increased Yields

- Improve Nitrogen Assimilation
- Increase Sucrose hydrolysis, Starch biosynthesis
- Increase O₂ availability
- Modify photosynthesis



Yield Gene

Control



Chimera

- Distinct genotypes growing side-by-side within the same plant.
 - **Periclinal** – occupies the outer layer of cells completely.
 - **Mericlinal** – occupies only a portion of the outer layer of cells.
 - **Sectorial** – occupies only a section of the stem extending through all cell layers.



PATTERNS OF GENETIC CHIMERAS WITHIN CLONES

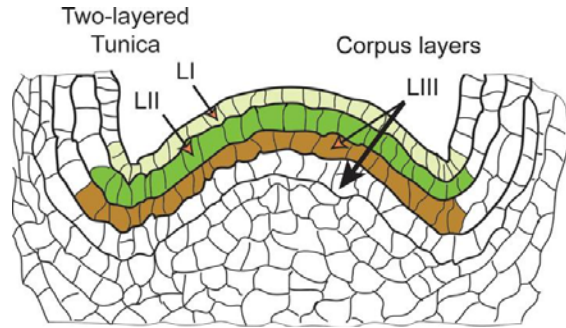


Figure 16-8 Chimeras: the dicot shoot meristem is usually organized into three distinct layers—L1, L2, L3. Typically, L1 gives rise to epidermal cells. L2 provides the next inner layer of cells and also the gametes. L3 cells become the inner most cells and the vascular system. Cells in the tunica (L1 and L2) divide anticlinally, whereas cells in the corpus (below L3) divide anticlinally and periclinally.

PATTERNS OF GENETIC CHIMERAS WITHIN CLONES

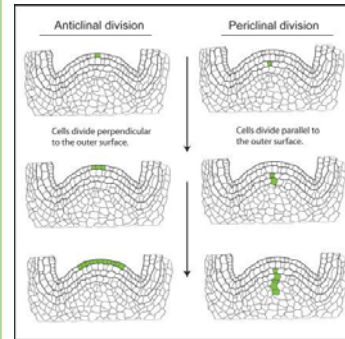


Figure 16-9 In anticlinal divisions the new cell wall plates of actively dividing cells form perpendicular to the shoot apex in Layer I, whereas in periclinal division, new cell wall plates form parallel to the shoot apex in cells dividing in Layer III and the corpus. The arrows indicate progressive division and growth of the shoot apex.

PATTERNS OF GENETIC CHIMERAS WITHIN CLONES

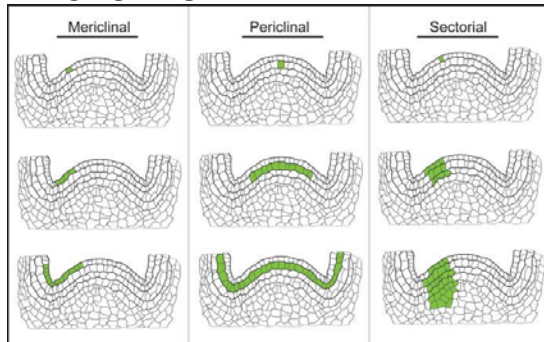


Figure 16-10 Mericlinal, periclinal, and sectorial chimera development. Only the periclinal chimera is stable and of horticultural importance. A segment of one or more apical layers is genetically different with mericlinal chimeras, while periclinal chimeras have one or more genetically distinct apical layers. In sectorial chimeras a segment of all apical cell layers is genetically distinct.

PATTERNS OF GENETIC CHIMERAS WITHIN CLONES



Figure 16-18 Chimeral reversion in (a) dogwood and (b) fuchsia from the desirable chimeral variegation back to a nonmutated, green form (arrow), or other mutation. The propagator needs to vigorously rouge-out these "off-types" and be sure to propagate cuttings with the desired chimeral variegation.

What causes phenotypic variations within clones?

- Environment by genotype interactions
- Ontogenetic aging (phase changes)
- Permanent genetic variation
- Infection by systemic pathogens
 - Viruses etc..



MANAGEMENT OF PHASE VARIATION DURING VEGETATIVE PROPAGATION



Figure 16-20 Foliage variation gradients within seedling trees and shrubs can be markers of juvenile and mature phenotypes. (a) Mature leaves of *Eucalyptus robusta* are alternate and lanceolate in shape, whereas juvenile (arrow) are opposite and have a silver-dollar form. (b) *Hedera helix* (English ivy) with juvenile and mature leaves and attached floral structures (arrow). (c) Needles of the basal juvenile part of many conifers tend to be acicular (sharp pointed), whereas needles on the (d) upper, more mature part of the juniper tend to be scale-like.



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Trueness-to-name vs Trueness-to-type

- **TTN** – implies that the plant conforms to the specific characteristics of the specified cultivar.
- **TTT** – implies the plants conform to the phenotypic expectations of the specific cultivar.





Trueness to Name How will you know?

- Visual inspection
- Isozymes – genetic variants of specific enzymes
 - identified by biochemical tests
 - used as genetic markers
- DNA-based marker technology
 - RFLP – restriction fragment length polymorphism
 - RAPD- randomly amplified polymorphic DNA






Trueness-to-type How will you know?


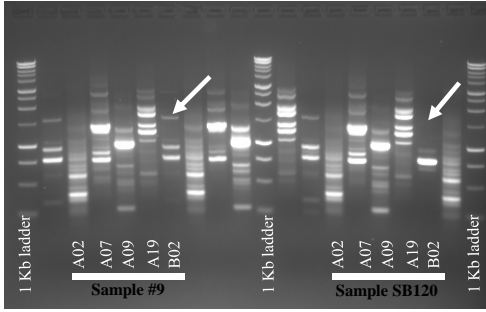

- **Visual Inspection**
- **Phenotypic Selection** – source selection based on phenotypic appearance of the source plant.
- **Genotypic Selection** – source selection based on phenotypic appearance of the vegetative progeny.
- **Vegetative Progeny test** - vegetatively propagating progeny to test their ability to reproduce the source plant.


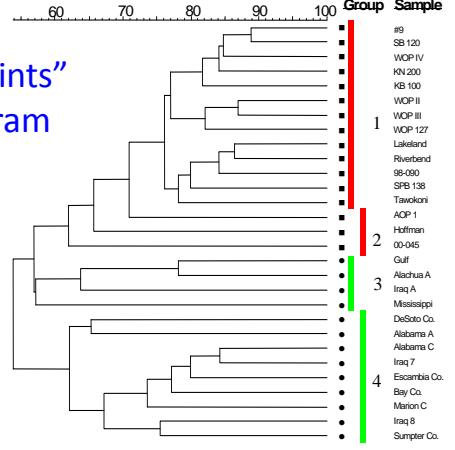
Visual Inspection


“Fingerprints” Randomly Amplified Polymorphic DNA Gel Images

“Fingerprints” Dendrogram

Group	Sample	
1	#9	
	SB 120	
	WOP IV	
	NV 200	
	KB 100	
	WOP II	
	WOP III	
	WOP 127	
	Lakeland	
	Riverbend	
2	98-090	
	SPB 138	
	Tawokoni	
	ACP-1	
	Hoffman	
	00-045	
	Gulf	
	Alachua A	
	Iraq A	
	Mississippi	
3	DeSoto Co.	
	Alabama A	
	Alabama C	
	Iraq 7	
	Eschambia Co.	
	4	Bay Co.
		Madison C
		Iraq 8
		Sumpter Co.



Conclusions

- Red leaf selections of *Imperata cylindrica* may result from more than one source.



Freedom From Pathogens

- Visual Inspection
- Culture Indexing
 - (fungi and bacteria)
- Virus Indexing
- Serology - ELISA
- Biochemical methods

Elimination of Pathogens?

- Selection of uninfected parts
- Shoot apex culture (micropropagation)
- Heat treatments
 - Hot water soaking, hot air
- Thermotherapy
 - Heat treatment over longer period of time (2 to 4 weeks)
- Growing seedlings

Propagation Source Management

- Commercial Plantings
- Commercial Nursery Crops
- Stock Blocks
- Clonal Selection and Pedigreed Production Programs
- Repositories, Botanical Gardens, and Plant Collections
- Quarantines and Movement of Vegetatively Propagated Material

Guidelines for selection

Obtain stock from:

- plants with a known history of production
- Plants that have been inspected

If genetic disorders are common:

- Conduct a vegetative progeny test
- Conduct frequent visual inspections
- Conduct test plantings and inspect for trueness to type



Pedigreed Production Programs

- Step 1
 - Identify individual plants within the clone that are genetically true and free of serious pathogens.
- Step 2
 - Maintain source plants in a protected Foundation block located to prevent reinfection.
- Step 3
 - Multiply source and distribute to the public.



NRSP-6 - United States Potato Genebank



To facilitate improvements in the potato of the future by promoting the use of valuable exotic genes found in wild potato germplasm.

Wild potato species represent a veritable treasure chest of genetic diversity for potentially useful traits that may someday be bred into new varieties.

5-fold approach: Introduction, Classification, Preservation, Evaluation and Distribution of potato germplasm.



PROPAGATION SOURCES AND THEIR MANAGEMENT

Table 16-1
COMPARISON OF CLEAN STOCK PROGRAMS IN REPRESENTATIVE CROPS

Phase	Potato (139)	Peanut (4)	Geranium (101)
I. Selection of sources	Clonal selection of "mother" plants from commercial plantings or as new cultivars. These are indexed and/or shoot-tip cultured to produce a nuclear source. Proc.	"Clonal selection" among individual trees. I. "Short index" either Stratigen or ELISA or both. II. "Long index" 6 to 8 indicators = 2 years. Provides nuclear source.	Stage I. Cuttings selected from source plants are visually inspected, culture indexed, and heat-treated for 4 weeks.
Visual inspections, indexing for virus or other pathogens, meristem culture, thermotherapy, hot water	I. In vitro cultured plantlets are multiplied to the desired number. Proc. II. Multiplied by sprout cuttings, which produces tubers for field production.	I. in vitro cultured plantlets are multiplied to the desired number. Proc. II. Multiplied by sprout cuttings, which produces tubers for field production.	Stage II. Meristem-tip cultured 5-11 plants are indexed and multiplied for 4 weeks (nuclear mother blocks). Stage III. 5-8 plants reindexed, grown for visual observation of performance.
II. Maintenance	Plants are called prebasic stock. Proc.	Two trees planted in a foundation block, isolated by distance or in a screenhouse. Annual Stratigen index or ELISA long index at other intervals. Trees are registered by location.	Stage IV. Plants from true-to-type and reindexed 5-11 plants are used to provide a nuclear block (2 months).
Visual inspections, roguing, indexing	I. Plants are transplanted to greenhouses where they develop three monthly harvests of minitubers. Proc. II. Tubers planted in field for 5 years of consecutive generations.	Annual Stratigen index or ELISA long index at other intervals. Trees are registered by location.	Stage V. Increase block where plants are multiplied and used to produce stock plants.
III. Multiplication and distribution	Field plantings: two steps I. Basic seed which involves 3 annual generations of propagation. II. Certified seed: 1 to 3 generations. Sold to potato producers.	I. To commercial nurseries; Mother blocks; nursery increase blocks; trees are registered by location. II. Commercial plants from mother blocks or increase blocks are certified.	Stage VI. Stock block from which commercial cuttings are sold.



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