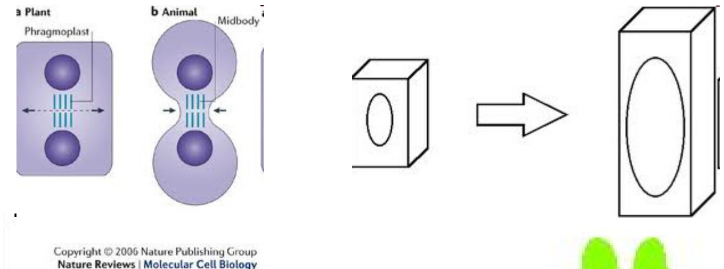
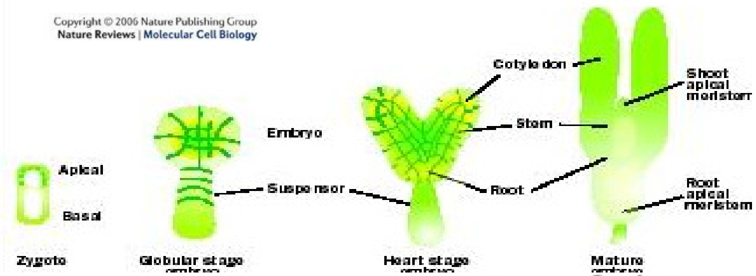
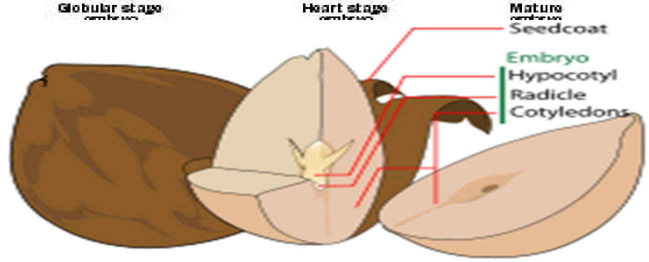
Sexual propagation in Plants



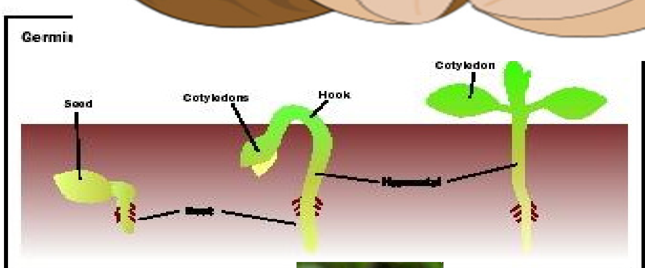
Cell division and expansion are particularly simple & fast in plants



Most embryos start with a fused cell called zygote- a fertilized ovule.



Most higher plants **start their life as an embryo contained in a seed** or a vegetative prop unit.

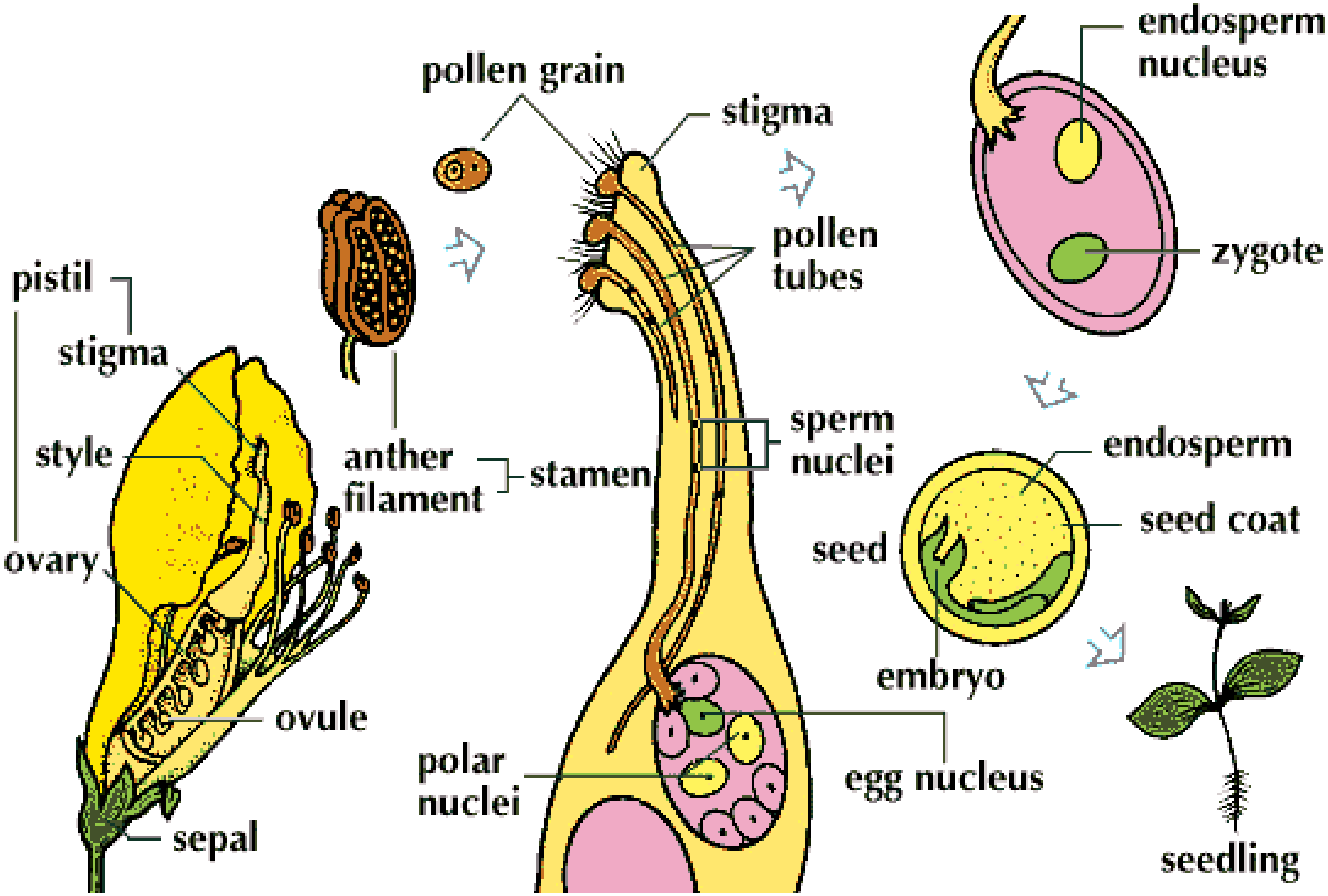


Most higher plants start their adult life **as germinating seedlings**

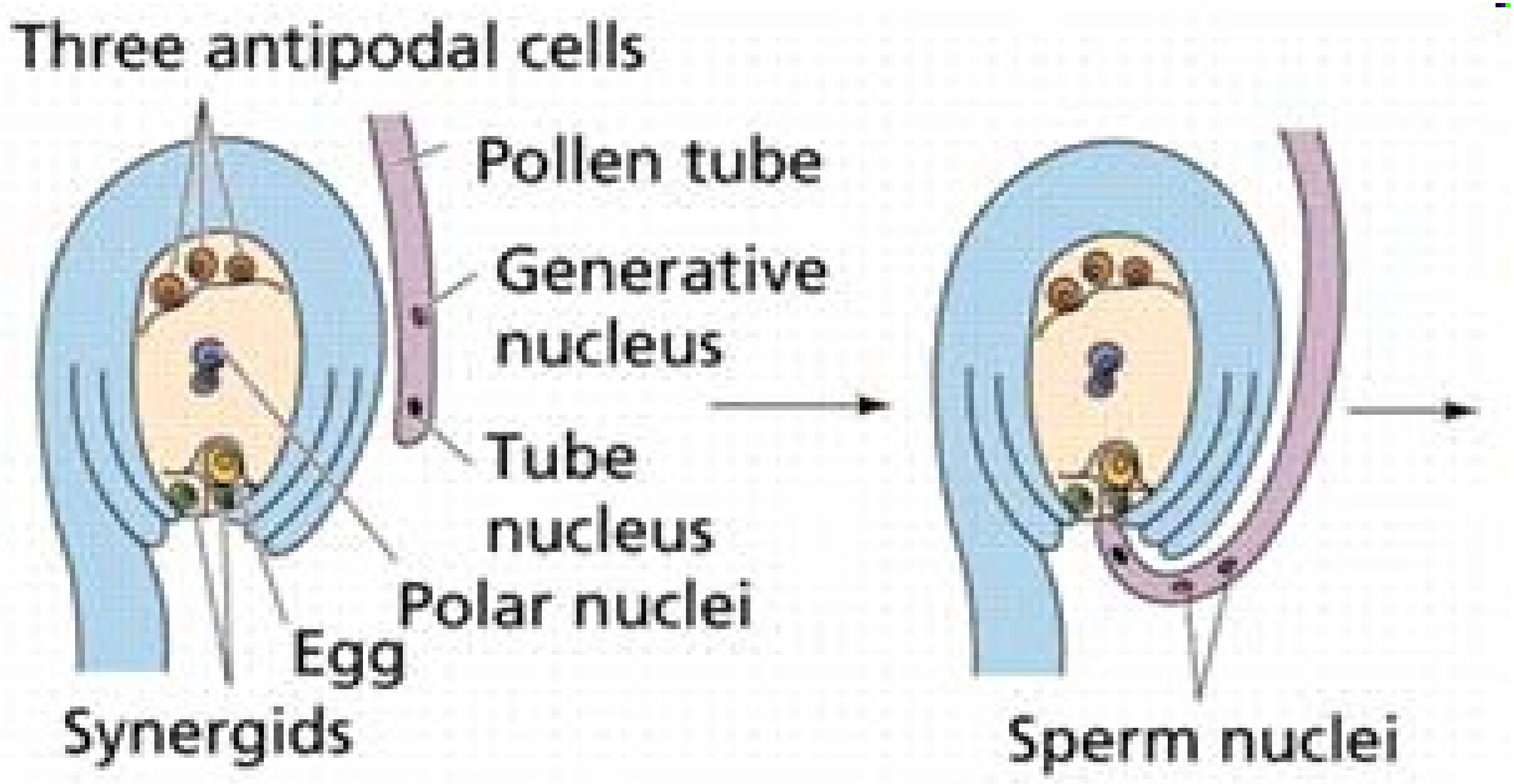
Plants reach sexual maturity by flowering & subsequent seed production

E. Haeckel's biogenetic rule" " Ontogenesis repeats phylogenesis"
➔ plant seedlings look very much alike in the monocots or dicots!

How higher plants reproduce!

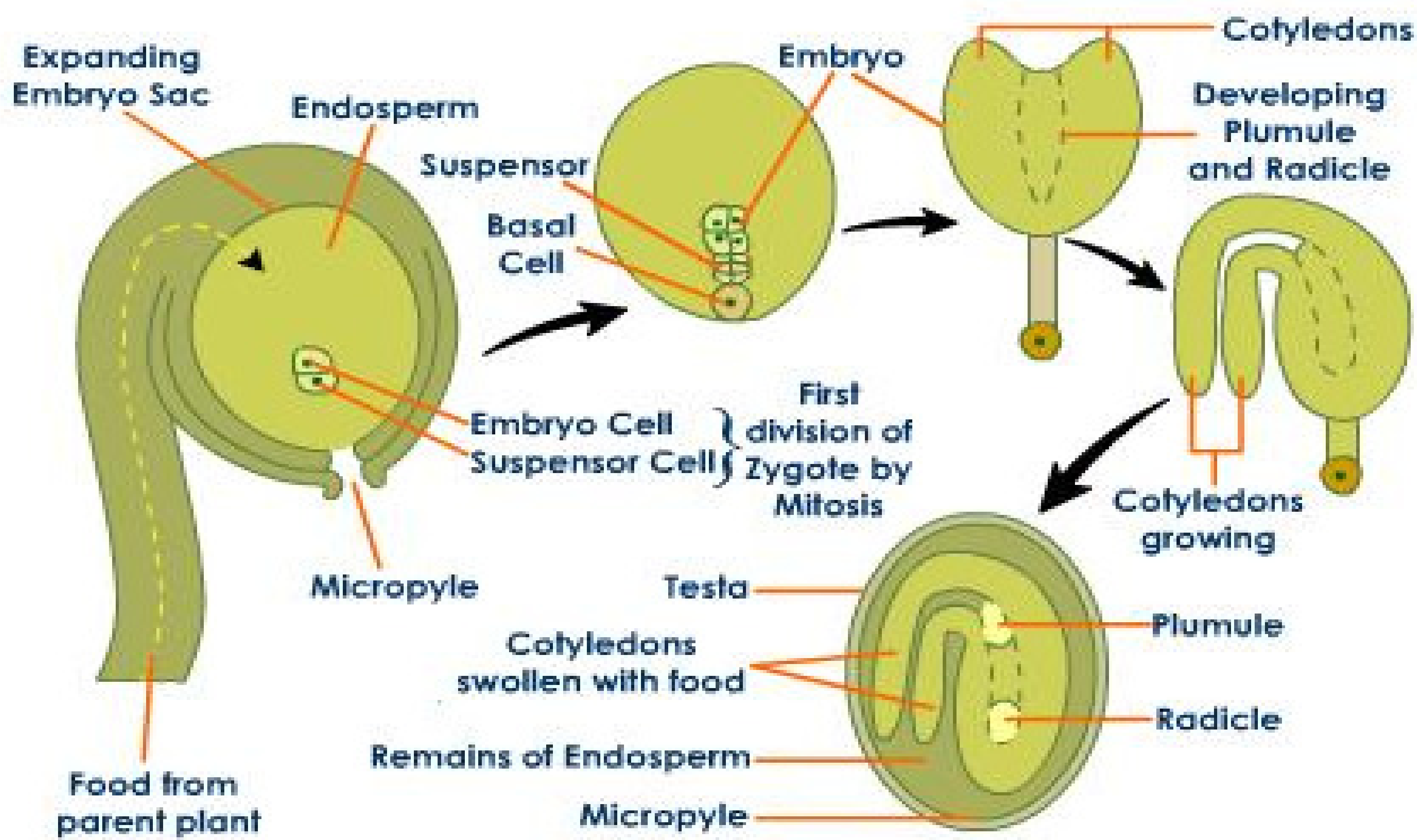


Embryo development: double fertilization



Fertilization in higher plants is different from lower plants and animals.
The pollen has 3 nuclei rather than one:
one to drive pollen tube expansion, one to fertilize the egg cell and form the embryo and one to fertilize the polar nucleus to form the endosperm

Embryo development in dicot seeds

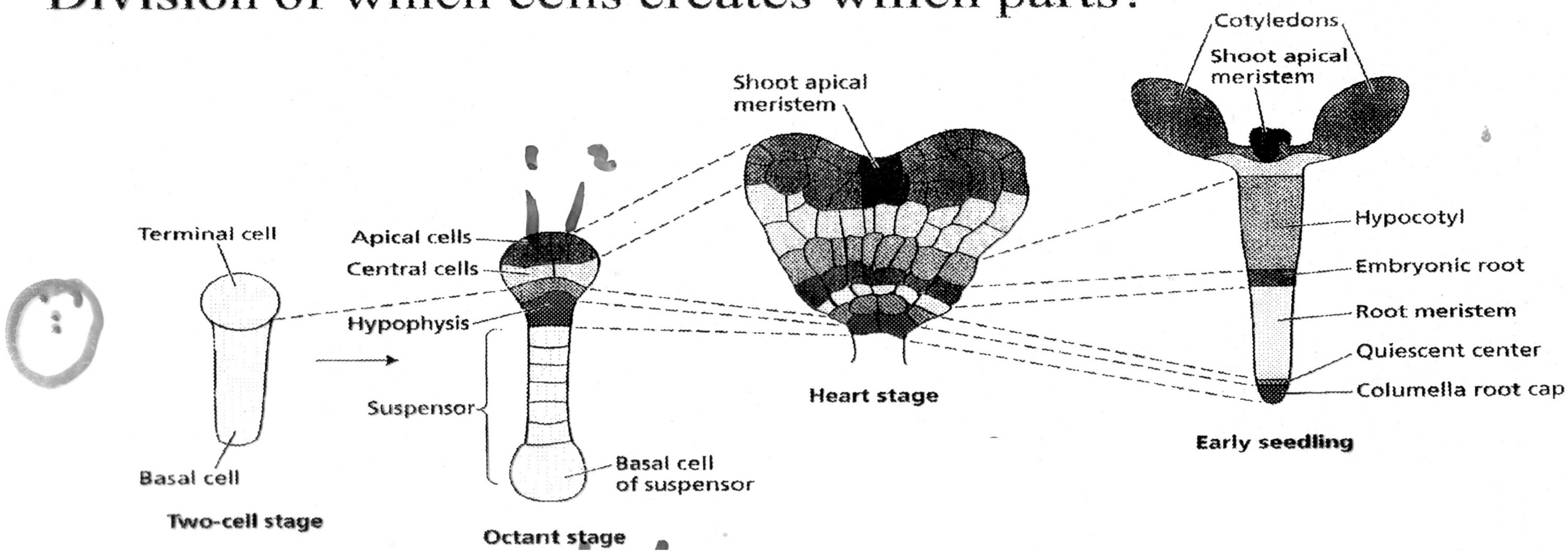


Note that early dicot seed still has endosperm, which gets digested to feed the swelling cotyledons as the new storage organ for food.

Cell division in seed development

1. First division establishes direction of axis (light, field)
2. apical region ➔ cotyledons, shoot apical meristem
   (expands during heart stage)
3. middle region ➔ hypocotyl, root, root meristem
   (expands during torpedo stage)
4. hypophysis ➔ contributes to root meristem, root cap

Division of which cells creates which parts?



Globular Heart Torpedo stage

Vivipary examples in Plants

Annuals survive (winter) only as seeds – if weather does not allow seed/fruit production ➔ extinction, they become perennials and continue as offshoots. e.g. Sedum annuum makes little rosettes of leaves
In alpine environments Poa anuua, Senecio, Viola tricolor become perennials

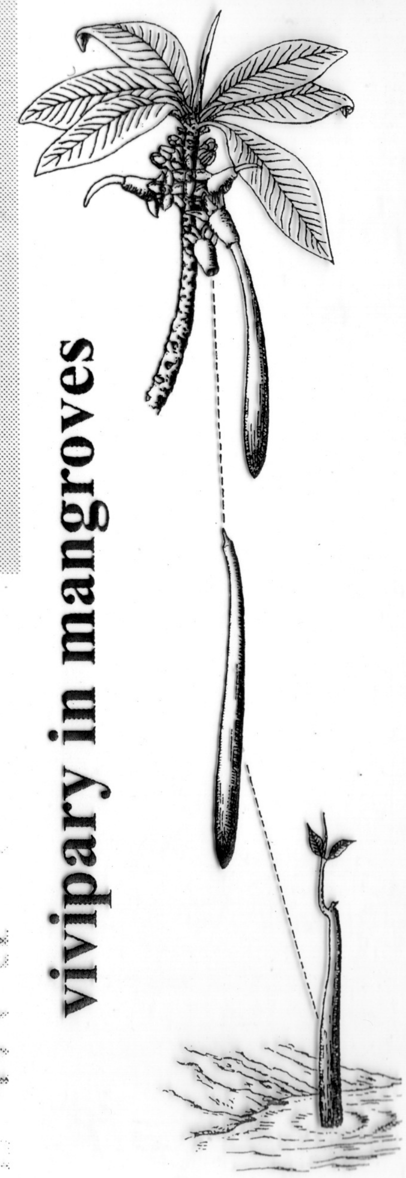
experimental removal of flowers turns annuals into perennials (death hormone)

Shade can cause Epilobium angustifolium to form long subterraneous runners

In grasses little bulbs or bulbils replace flowers and fruits: Poa alpina in the NW Poa bulbosa -- this is not seed germination but production of asexual units

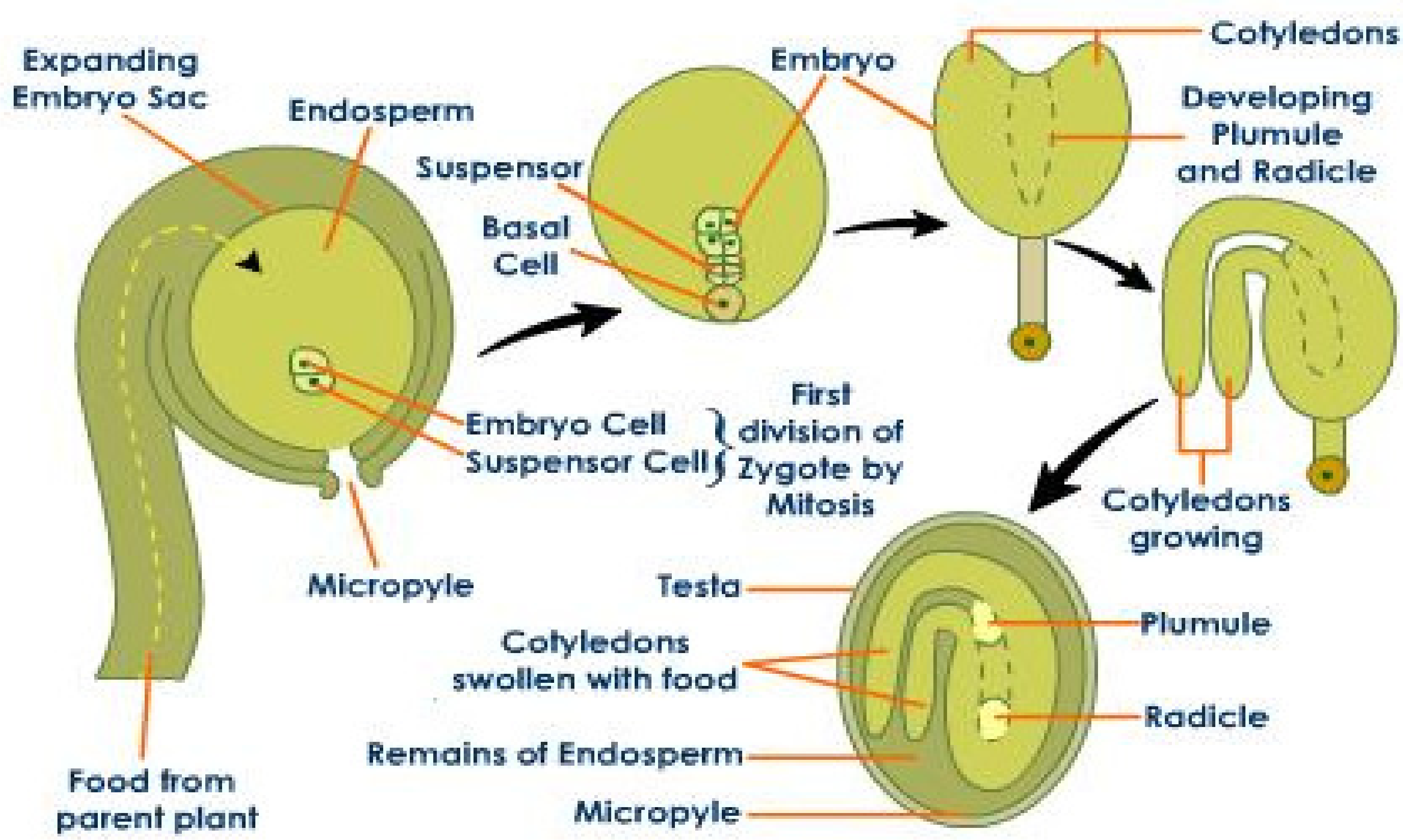
Saxifraga nivalis Saxifraga cernua Poa alpina
showing viviparous behavior
with: leafy rosettes (2) bulbils (4) bulbils (9,30)

bud-like offshoots in
axils of Ranunculus ficaria



vivipary in mangroves

Embryo development in dicot seeds



Note that early dicot seed still has endosperm, which gets digested to feed the swelling cotyledons as the new storage organ for food.

Plant dormancy – Seeds

Plant embryos are always put into dormancy at the end of the embryogenesis to prevents germination on the mother plant ➔ **vivipary**

Def.: Viable seeds that do not germinate are said to be *dormant*.

When a mature, viable (embryo cells alive) seed fails to germinate under favorable conditions (water, temps), it is dormant. **2 types of dormancy ➔**

1. **seed coat dormancy** or **external dormancy** is caused by the presence of a hard seed covering or seed coat that prevents water and oxygen from reaching and activating the embryo.
2. **embryo dormancy** or **internal dormancy**, is caused by inhibition of the embryo which prevents it to germinate under increased hydration

In the dehydrated (< 10 % water) state of dormancy, the embryo can survive extremely hot or cold temperatures (300 C for a short time), radioactive radiation is not causing genetic mutations.

➔ Therefore **annuals survive adverse environments like winter or drought as seeds**

How to overcome seed dormancy!

Wild plants have exceeding long periods of dormancy, **domesticated plants do not**. Dormant seeds were abolished during the selection process of harvesting and sowing the next year. There are several ways to overcome the dormancy of seeds.

Scarification is artificial damaging of seed coat to allow access of water. Use of file (mechanic sc.) or 10 min soak in con. Sulfuric acid (chem. S)

Stratification: cold treatment simulating the winter frost. Seeds are mixed together with moist sand or peat moss and stored for a few weeks in the refrigerator (4 C or 40 F). This treatment starts a process that breaks down abscisic acid and inhibitors of embryonic development.

Seed Banking refers to the fact that some plant species (weeds, Scots broom) have many generations of seeds in the soil. A variable length of the dormancy create seed banks, which allow them to germinate even if the previous years did not set seeds or even if the plant was destroyed years before.

Sometimes dormancy of plant embryos is not inforced - vivipary

The generation of embryos in higher animals is a strictly sexual process **In plants this would be through spores and seeds. In plants – unlike most animals - however, embryos can also be made from normal body cells too**. Such **embryoids** are generated naturally in plant with viviparous embryos at their leaf, stem & root surface:

Red Mangrove (*Rhizophora mangle*)

Kalanchoe daigremontana, Asplenium walking fern Tolmiea menziesii, bulbils of Dioscorea, the bomb-like seedlings of Rhizophora – the mangroves

Other Vivipary examples in Plants

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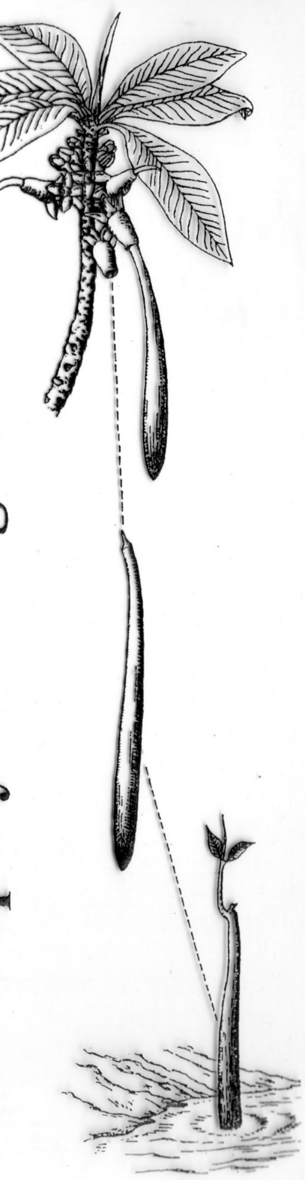


Plant dormancy –Trees & Buds

Plant embryos are always put into dormancy at the end of the embryogenesis to prevents germination on the mother plant ➔ **vivipary**

Tree dormancy: Trees that have dormancy needs too. ➔ leaf abscission and dormant buds. **Deciduous plants** will lose their leaves; **evergreens** will curtail all new growth.

if a Japanese Maple (*Acer palmatum*) is given an "eternal summer" through exposure to additional daylight, it will grow continuously for as long as two years. Eventually, however, a temperate climate plant will automatically go dormant, no matter what the environmental conditions..

Going through an "eternal summer" and growth is stressful to the plant and usually fatal. The fatality rate increases to 100% if the plant does not receive the necessary period of cold temperatures required to break the dormancy. Most plants will require a certain number of hours of "chilling" at temperatures between about 0 ℃ and 10 ℃ to be able to break bud dormancy

How plants survive the winter!

Annuals survive the winter as **dormant seeds, biennials** as buried storage roots, bulbs or shoots (rhizomes), **perennial plants** survive with their shoots in the open; have **new buds** ready in the fall.

Seeds & buds form next generation of full plants or shoots only. Bud is **embryonic shoot with short internodes & protective bud scales** as leaves. Bud dormancy develops before the old leaves senesce and fall. Shortening of day length induces dormancy in buds.

How to overcome bud dormancy? .

1. Dormancy is broken in s leafless trees by **rising day length**: birch, , larch yellow poplar, red oak *scales are light-sensitive, keep them indoors in constant light or try method 3 below :Molisch*

2. Bud dormancy can be overcome **by low temps** apples need 1000 hours at 7C, lilac branch inside warm greenhouse will stay dormant, branches outside house will overcome dormancy

3. Immersing branches in warm water baths at 40-55 C for a minute

Vegetative Propagation

The amazing feature of plants (fungi, bacteria, lower animals) to complete or regenerate a new plant from as little as a few body cells. It implies that ***plants can sense whether they are complete or not*** and also recognize what parts are missing. How this is done we do not know.

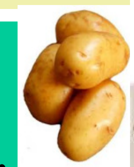
Involves non-sexual reproduction through regeneration of tissues & plant organs from one plant part or tissue.

Vegetative propagation is easier & more reliable than seed propagation since it avoids chance changes due to parental variability.

Enables the **cloning of genetically identical material** & continuation of a desirable clone not able to make seeds

Vegetative Propagation – play it safe

Plants consider vegetative propagation the safer method . When they have to choose between asexual and sexual propagation, the clearly vote for the first one. Examples are plants that do rarely produce flowers: One reason for this behavior is that the assimalated organic substances are drawn to the geophytic organs rather than the flower



Potatoes *Solanum (Lycopersicum) tuberosum*. In large potato fields you find very few plants that are flowering.

Horseradish *Cochlearia armoracia* flowers but hardly ever produces seeds since the growth of storage roots has absolute priority.

Buttercups of *Ranunculus ficaria* flower in early spring but hardly produce any fruits. They propagate by small visible bublets that form near leaf nodes at the stem.

Jerusalem artichoke, Topinambur *Helianthus tuberosum*. Flowers in late summer but never succeed making seeds store inulin in tubers

Vegetative Propagation

There are various possibilities of vegetative propagation, but all are either natural methods or horticultural (man-made) methods.

Natural methods:

Runners,
Bulbils,
Bulbs
Corms,
Viviparous leaves

Man-made/horticultural methods:
Stem cuttings, Leaf cuttings
Root cuttings
Layering Grafting

Improvements of offspring number by slices of tubers, korms, rhizomes

Vegetative propagation is based naturally on somatic embryos, buds or when man-made in addition to above also on wounding, darkening, etc.

Vegetative Propagation --- Runners & Stolons

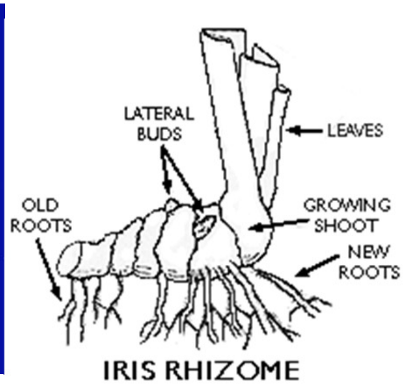
Runners = Stolons are horizontal stems which grow at the soil surface or below it while forming new plants at the ends or at the nodes.

Strawberry *Fragaria* spider plant *Tradescantia* runners are separated

Fleabane *Erigeron* Saxifraga runners potato tubers form on runner

Vegetative Propagation --- Rhizomes

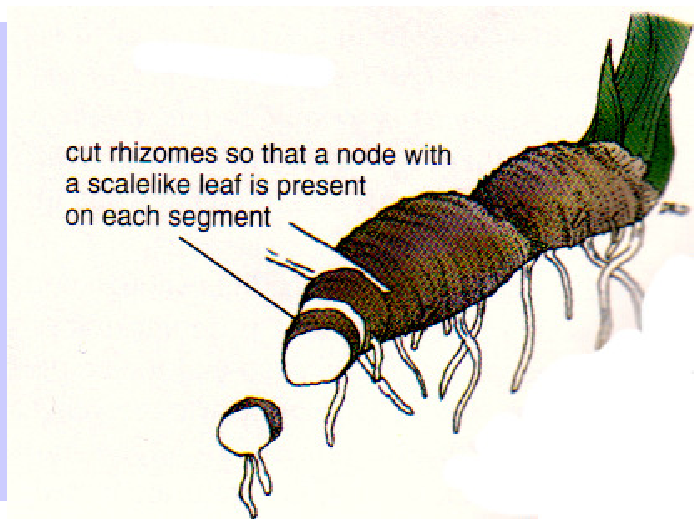
A **rhizome** is a horizontal stem (it carries nodes) that is usually found underground. Rhizomes have nodes & short internodes; send out roots from the bottom & shoots from the top of the nodes.



Iris rhizomes (3 views) ginger rhizome

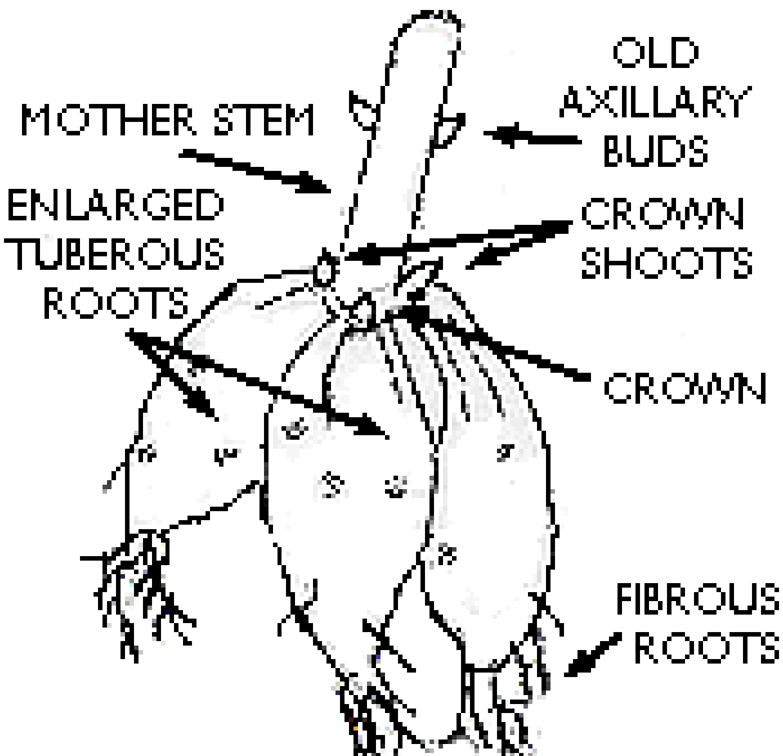
Note the etiolated appearance & thin papery skin of many rhizomes.

Ginger, Venus Flytrap, hops, Johnson grass, Bermuda grass. Some plants like ferns have rhizomes as the only stem. Rhizomes are used by gardeners to propagate plants like hops, asparagus, ginger, irises, Lily of the Valley, Cannas, sympodial orchids.

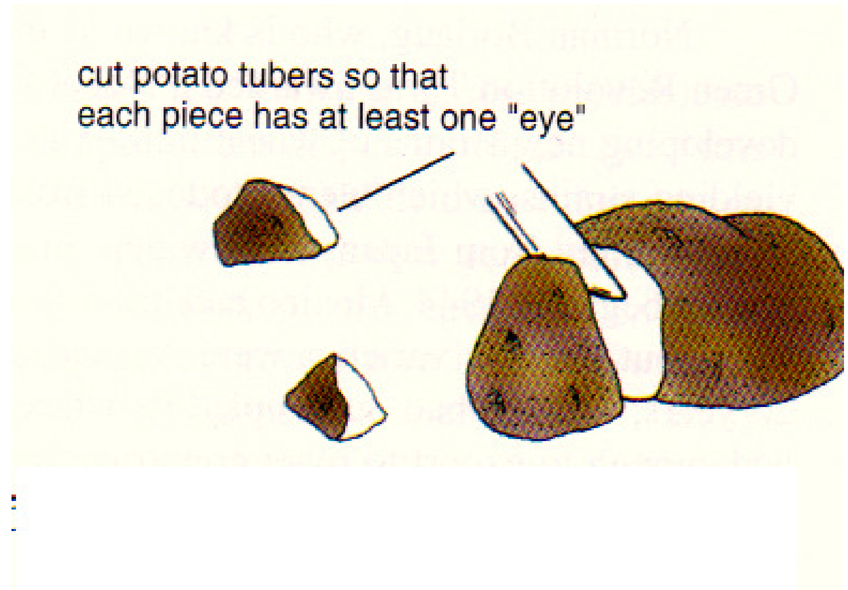


Vegetative Propagation – root tuber

A tuberous root consists of enlarged fleshy root tissue because, it is the primary storage tissue. Growth arises from buds at the top (crown) of the root mass. Examples include dahlia, anemone, and Ranunculus.



TUBEROUS ROOT OF DAHLIA

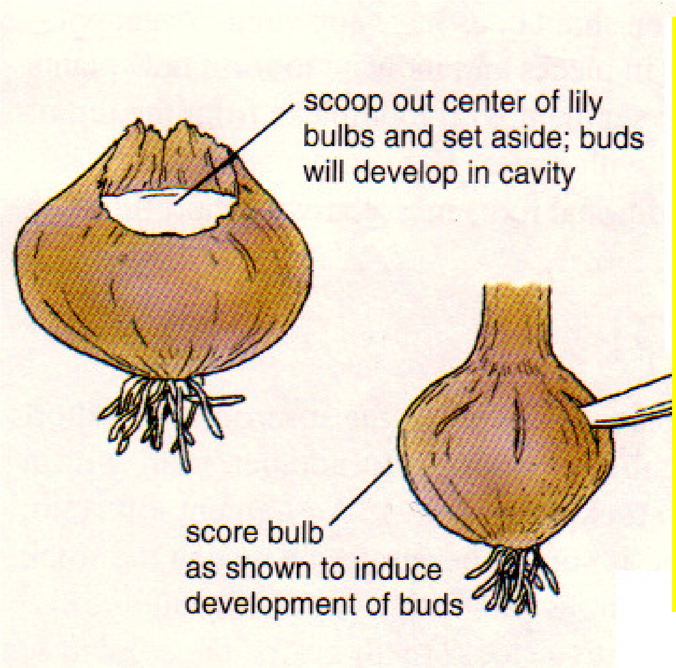


Not a root tuber but a stem tuber that derives from a thickening of the stolons or runners of the potato.

Vegetative Propagation – Bulbs & Perennials

A **bulb** is a short underground vertical shoot that has thickened leaves used as food storage organs by a dormant monocot plant. A modified stem forms the base of the bulb, and plant growth occurs from this basal plate. Roots emerge from the underside of the base, and new stems and leaves from the upper side

Some epiphytic orchis (family Orchidaceae) form above-ground storage organs called **pseudobulbs**, that superficially resemble bulbs.



Gardeners can increase the number of plants propagating from one bulb by wounding it to induce embryonic plants (buds) for propagation.

Tulip (top) & lily bulbs ➔

All plants that form true bulbs are **perennial monocotyledons**: onion, garlic, and other alliums, lily, tulip, and many in the lily family ; *Amaryllis*, *Hippeastrum*, *Narcissus*, and other members of the *Amaryllidaceae, Iris family*

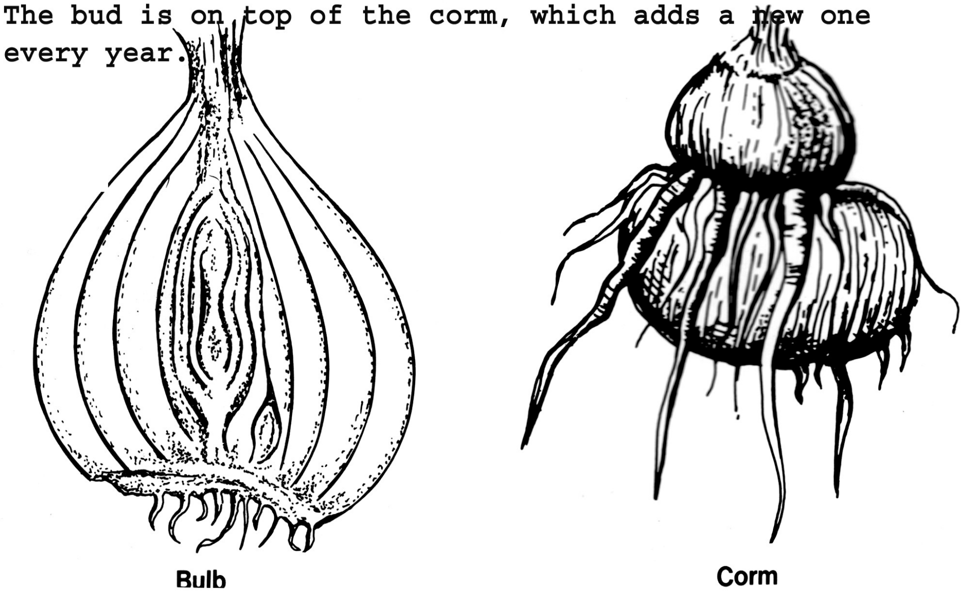
Vegetative Propagation – Corm

A corm is a short, vertical, swollen underground plant stem that serves as a storage organ to survive winter or summer drought and heat. A corm has one or more internodes with at least one growing bud, surrounded by protective papery skins or tunics.

Corm of taro *Colochasia esculenta*

Corm of *Gladiolus showing the formation of small cormels at the end of short stolons*

Bulb and corm are structurally very different. A bulb has a base plate which is the short stem.
A corm is a swollen stem base, solid mass of starchy cell
The bud is on top of the corm, which adds a new one every year.



Vegetative propagation is based naturally on multiplication of ← small cormels on runners

Vegetative Propagation – Bulbils

Some lilies form small bulbs, called **bulbils** in their leaf axils. Several members of the onion family, *Alliaceae*, including *Allium sativum* (garlic), form bulbils in their flower heads, or even instead of the flowers. The so-called (*Allium cepa* var. *proliferum*) forms small onions large enough for pickling.

Allium cepa

Allium sativum

Poa bulbosa bulbous bluegrass

← Bulbils of Dioscorea air potato yams

Poa bulbils magnified →

Vegetative Propagation – Division

The simplest method of vegetative propagation is division.

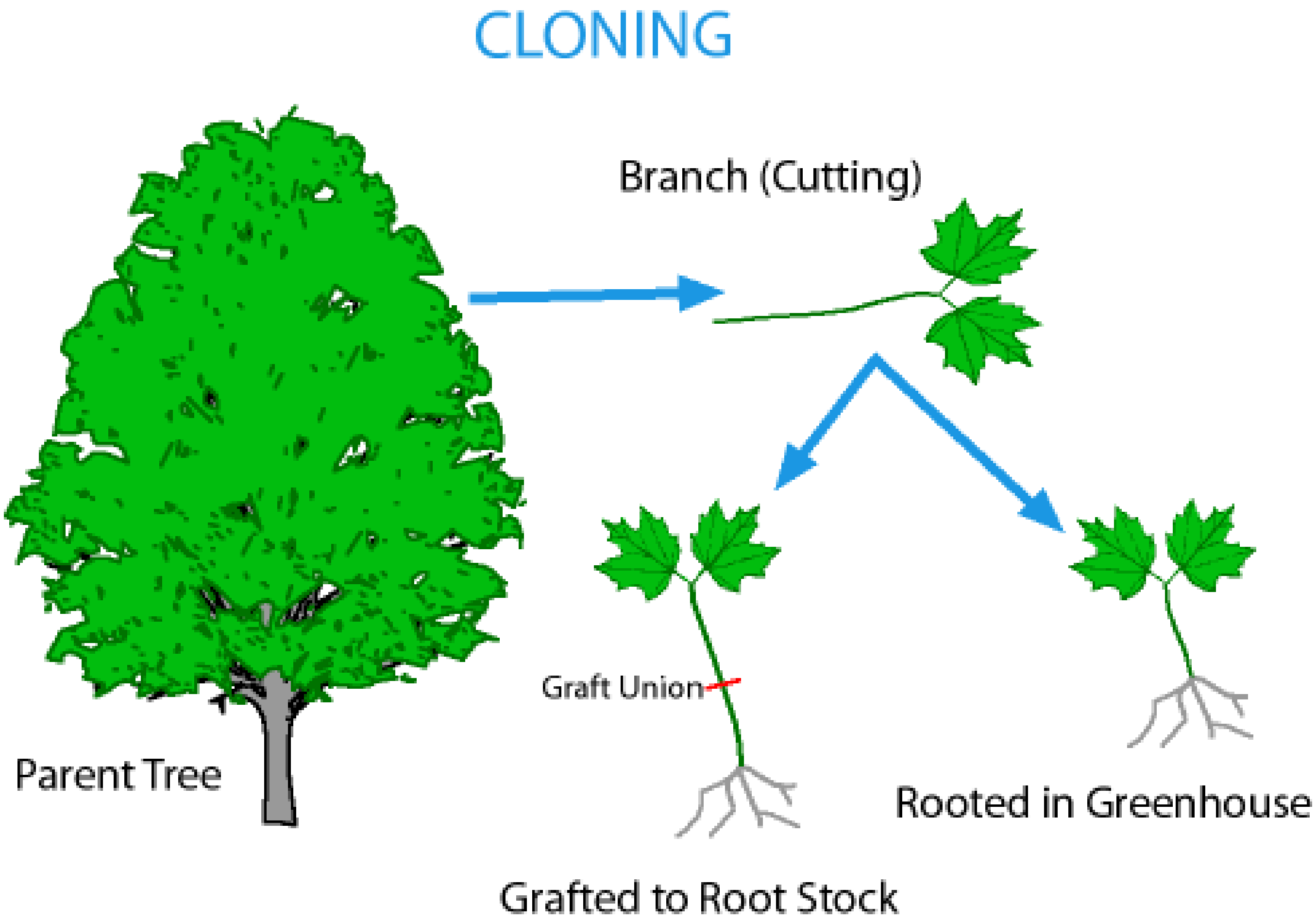
Propagation by division assures the new plant will be an exact match with the original.

Division is an inexpensive way to create extra plants for swapping with friends.

Fall is a good season to divide or split many perennials.

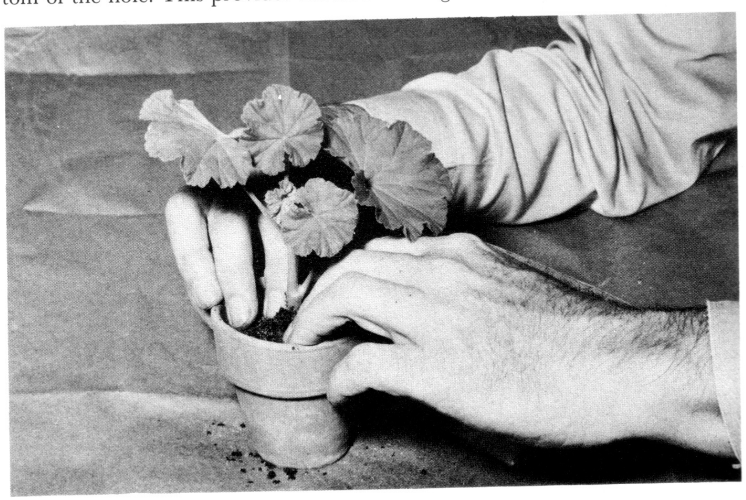
Vegetative Propagation – Cuttings

Plant cutting, also known as striking/cloning, is a technique in which a piece of the source plant is placed in a suitable medium such as moist soil, potting mix, coir or rock wool. The cutting lacks one organ & must produce new roots or stems.



Vegetative Propagation – Stem Cuttings

A 3-inch pot is filled with standard seedling and cutting mixture, a hole is made with the blunt end of a pencil, and a little sand is poured into the bottom of the hole. This provides the free drainage which is desirable.



Insert cutting, press down on soil,water,=> under plastic bag

Stem cuttings need to be rooted, June to August is right time to do that (3 –9 weeks time).

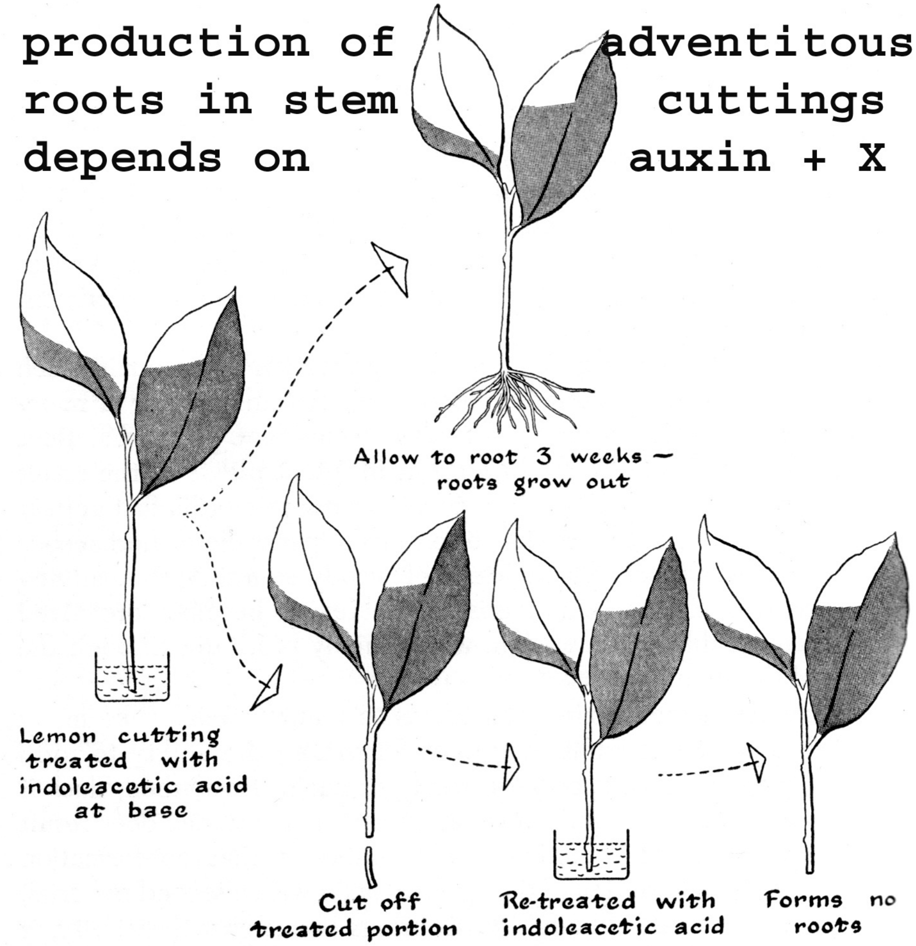
The danger is dehydration, so shade or a mini-greenhouse (= plastic bag over pot)

You can root stem cuttings in water but it is better to darken the under-water part.

In soil good drainage is also necessary.

Softwood cuttings of shrubs and trees, in July Azaleas, barberries, boxwood, Viburnum, Juniper, Rose, Thuja etc.

Vegetative Propagation – Stem Cuttings



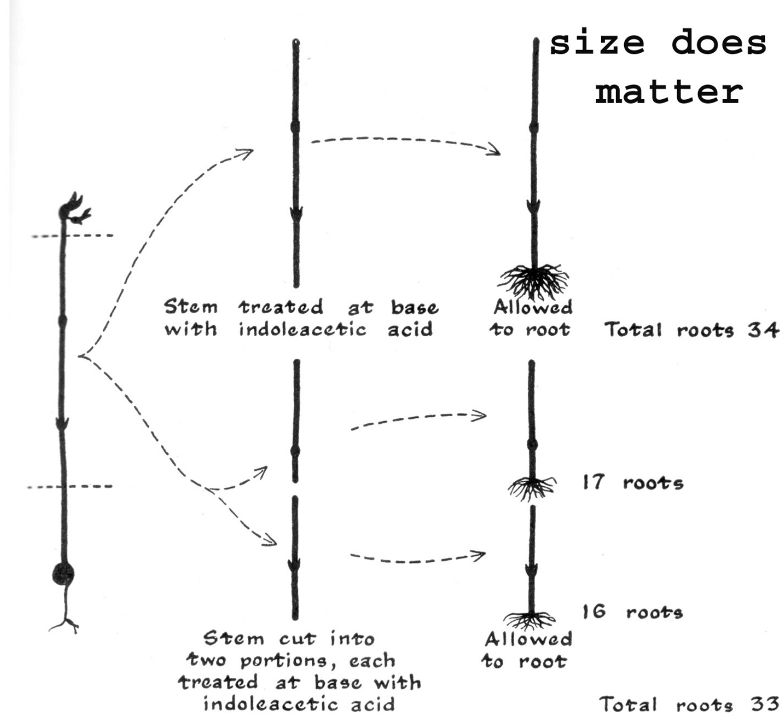
The production of adventitious roots depends not only on auxin but on another factor or factors which are normally present in many stems and which may be mobilized to the site of auxin application. (Adapted from Cooper, Plant Physiol., **10,** *1935, p. 791.)*

Rooting of stem cuttings depends on

(1) the size of the stem (see pea exp)

(2) auxin + (3) a wound factor x

that can be removed by a second cut.
➔ cut stem only once !!!

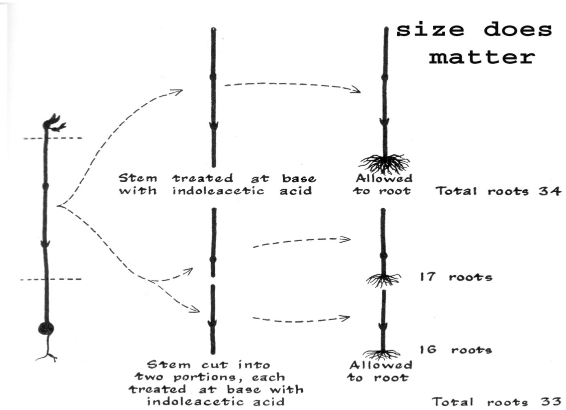


The maximum number of adventitious roots formed by a cutting as the result of auxin treatment depends on the amount of other root growth factors which the cutting contains. This is determined in the pea by the size of the cutting. (Adapted from Went, Plant Physiol., **13,** *1938, p. 69.)*

Hormone Auxin plus something else X

Vegetative Propagation – Plant cells need neighbors to grow

If you want to propagate trees through excised branches, these should be longer than 7 cm and should carry buds.



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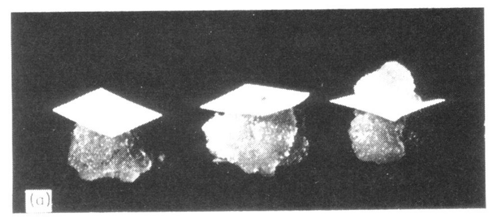
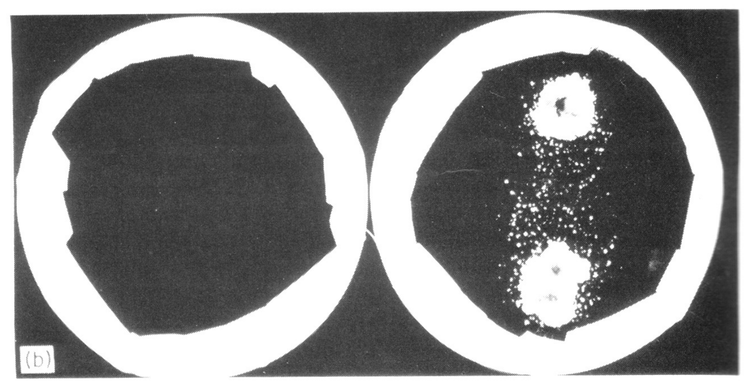
If you want to propagate a callus you need more than one cell or better go with a **nurse culture**





Growth & division of a single isolated cell on top of a piece of filter paper contacting a nurse callus (top)

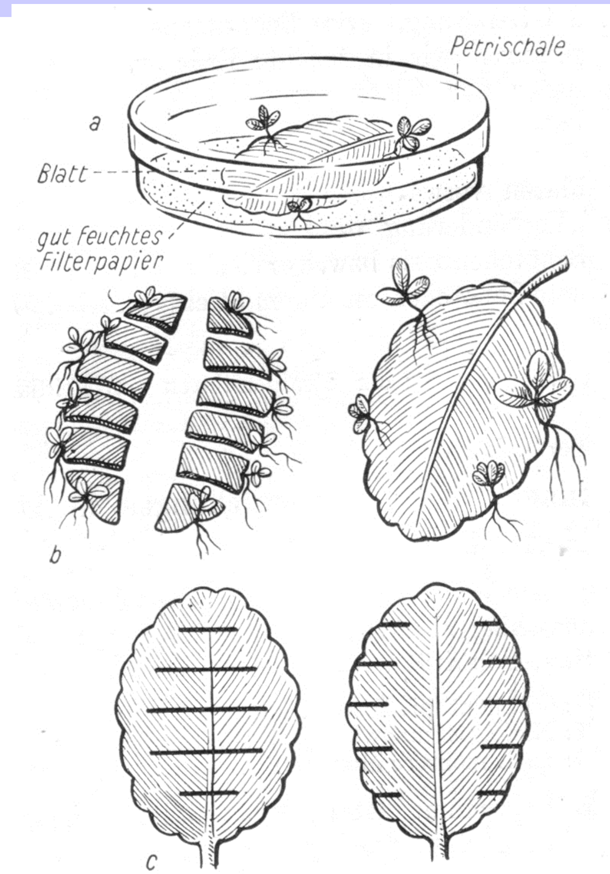
If you want to propagate an embryo in-vitro you better make sure that that it reached the octet stage or you will fail. Nobody succeeded cultivating zygote in in-vitro so far.

Vegetative Propagation – viviparous leaves

Vivipary is the production of living seedlings instead of flowers. We find this in many plants from

Red Mangrove (*Rhizophora mangle*)

Walking fern, Tolmiea menziesii, Rhizophora, Kalanchoe , Poa all show **somatic embryognesis** at the leaf margins. This can be improved by wounding the leaves in Kalanchoe & Begonia



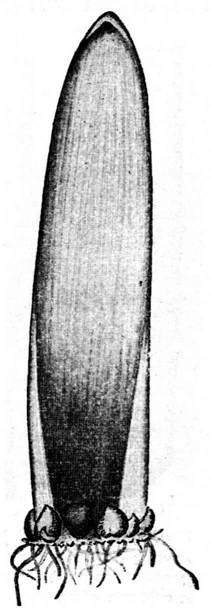
Kalanchoe leaves with piggy-back seedlings from peripheral leaf-born embryos

Vegetative Propagation – Leaf Cuttings

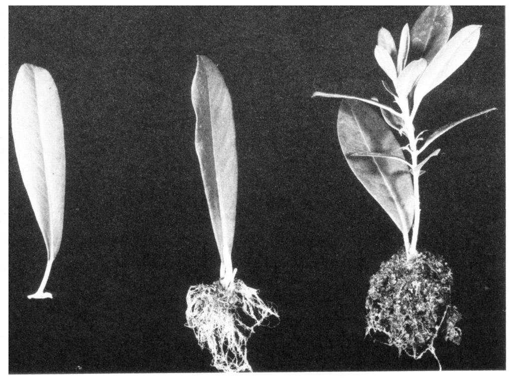
Ideal for viviparous plants like ***Tolmiea menziesii,* water lilies *Nymphaea daubeniana, Kalanchoe*, walking fern, *Sedum, Echeveria*.** But also ***Hyacinthus, Begonias, Pepperomia & Rhododendrons*** can be tried

Begonia leaf on soil
also cuts at midveins
may help

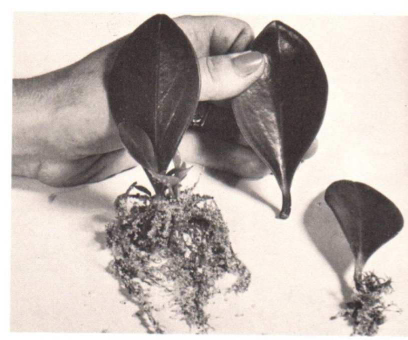
A leaf of *Begonia Rex* is cut with 1-inch leafstalk (petiole) and the main veins severed. The stalk is inserted in the sand so that the entire leaf lies flat on the rooting medium. It may be necessary to put two or three stones on the leaf to hold it down, or, if you have any hairpins, you can pin it down with them.



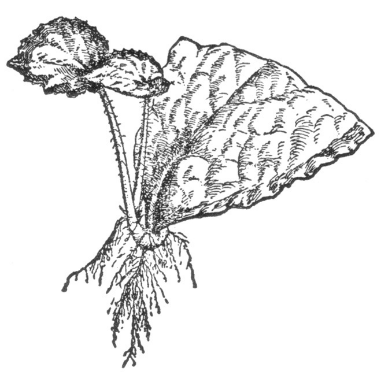
Hyacinthus
leaf after
2 months
in wet sand



eaf-bud cutting of Rhododendron
after 4 weeks, 5 months

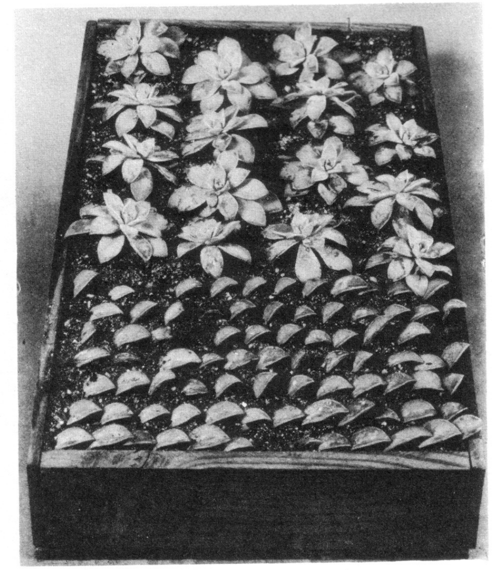


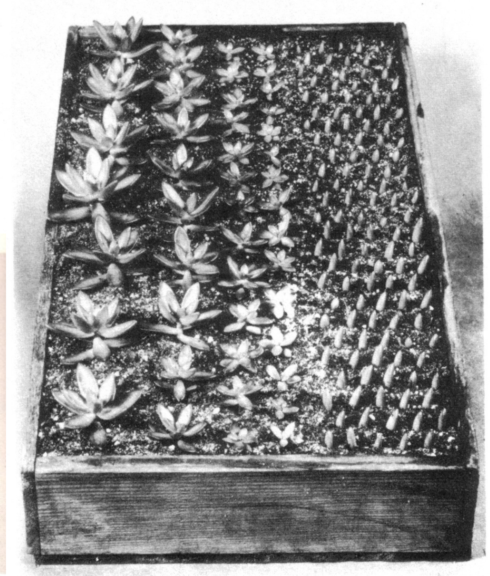
Peperomia obtusifolia, with leaf cuttings in various stages.



Begonia leaf cutting
wedge cut with midvein

If preferred, the leaf can be cut to wedge-shaped pieces, each containing a main vein.





Echeveria (above) &
Sedum leaves (below)

Vegetative Propagation - Leaf Cuttings

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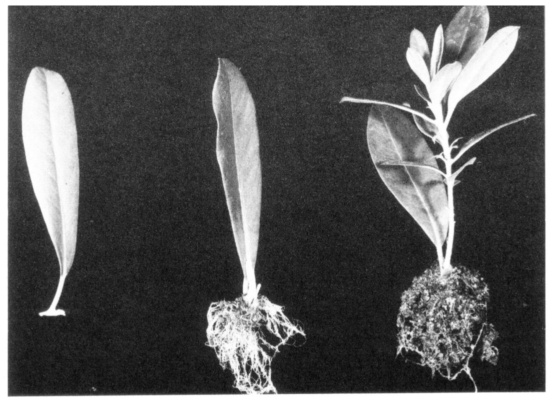
also cuts at midveins
may help

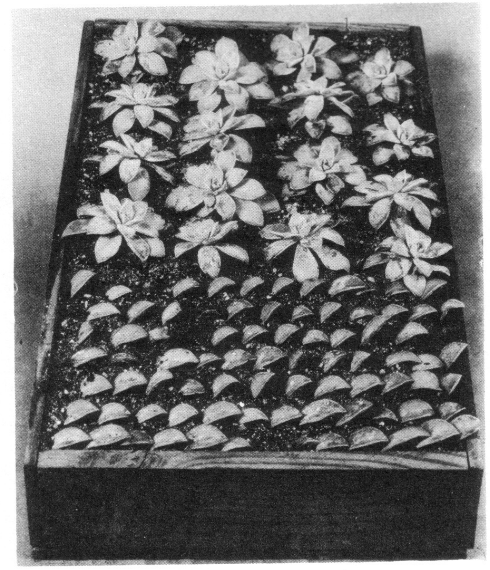
A leaf of *Begonia Rex* is cut with 1-inch leafstalk (petiole) and the main veins severed. The stalk is inserted in the sand so that the entire leaf lies flat on the rooting medium. It may be necessary to put two or three stones on the leaf to hold it down, or, if you have any hairpins, you can pin it down with them.

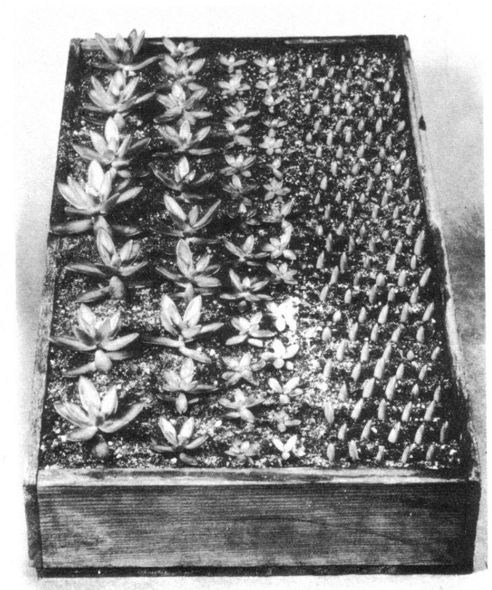


Leaf-bud cutting of Rhododendron
after 4 weeks, 5 months

Begonia leaf cutting
wedge cut with midvein

If preferred, the leaf can be cut to wedge-shaped pieces, each containing a main vein.

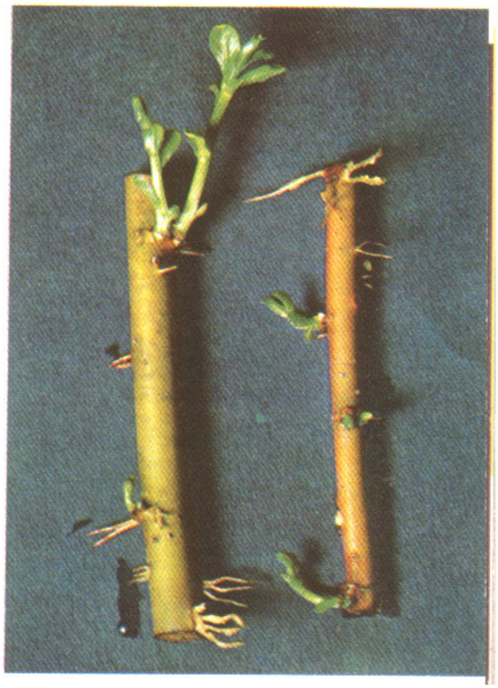




Echeveria (above) &
Sedum leaves (below)

Polarity

Like magnets also plants have a clearly defined polar structure with the apical end producing leafy shoots and the basal end making roots. The buds sense the incompleteness & also remember their pole / polarity.

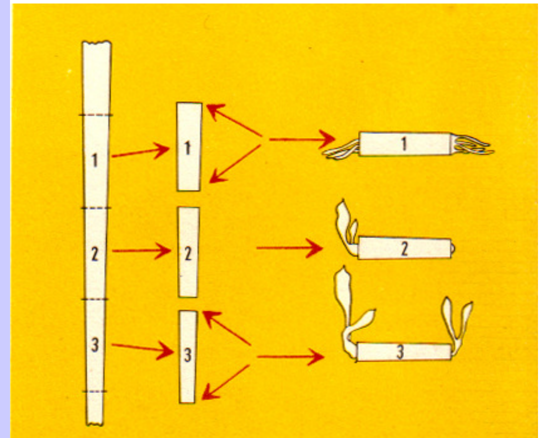


apical ends are recognized by their tendency to make leafy shoots, basal ends to make roots. This is independent of their actual orientation to gravity.

Polarity does not change when gravity vector is ← inverted.

Only plant cells that are temporarily without fixed polarity are the zygote & callus or tumor cells.

One can force the basal end to produce leafy shoots by adding a gypsum prop to apical end or the apical end to make roots by adding auxin to it.



Taraxacum root slices

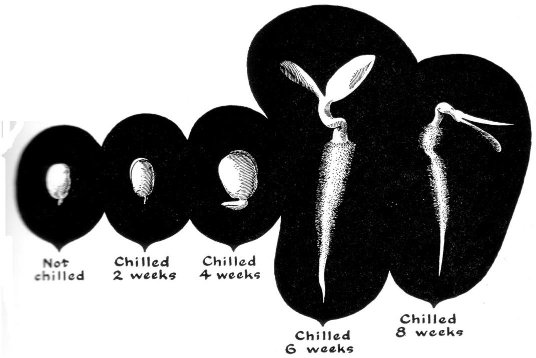
1. adding auxin to apical cut => root growth

2. normal control => appearance of leafy shoot precedes roots

3. Destruction of auxin by ethylene chlorohydrin causes appearance of shoots on both ends

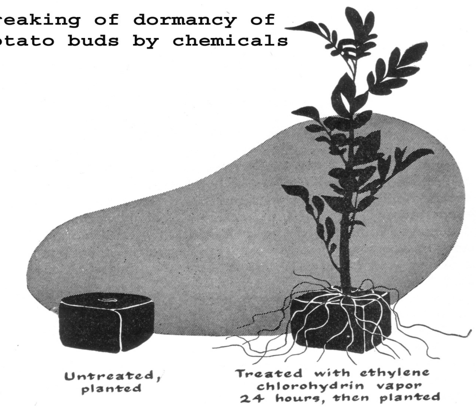
Vernalization of seeds & buds!

Vernalization means to turn a somebody into spring mood. In plants this is done by exposing them to cold in a simulation of winter. As soon as they leave the refrigerator they are turned on to grow.

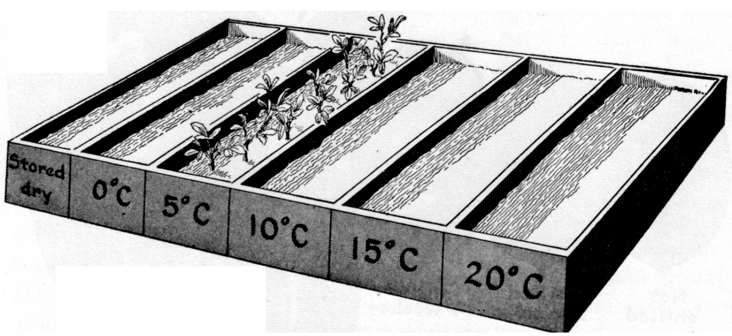


Dormancy of Sorbus (ash) Rosaecea is overcome not beffore it has been chilled for 6 six! weeks

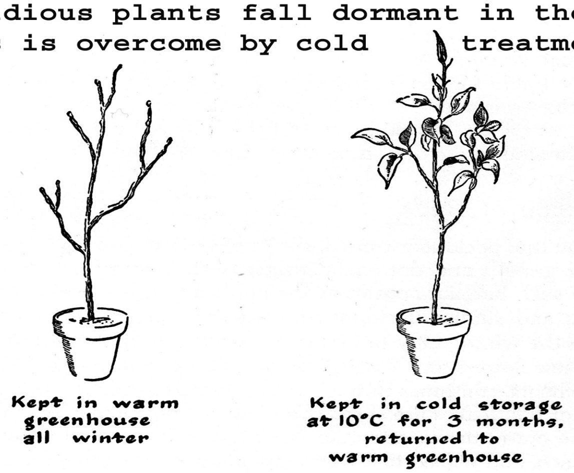
Breaking of dormancy of potato buds by chemicals



How to determine optimal temperature for breaking the dormancy of cold-requiring seeds of rose!



Decidious plants fall dormant in the fall This is overcome by cold treatment!

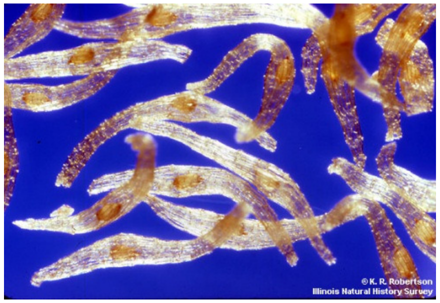


Vernalization is misleading term: It is not spring but winter that is simulated

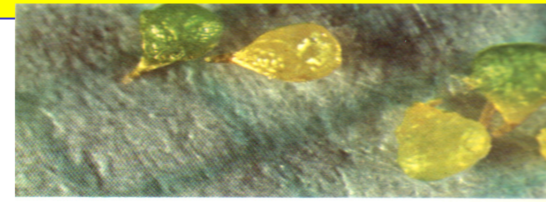
Important to find both the right temperature & right time.

Let us grow …. Seeds… in culture. What do we learn?

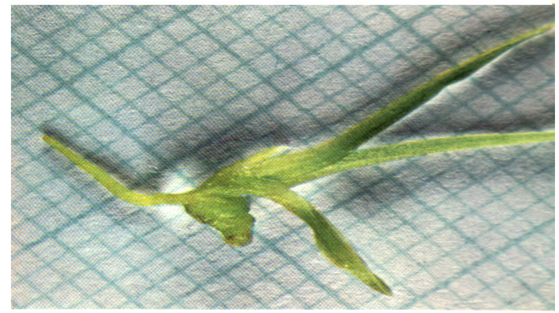
Seeds can be grown in aseptic culture since contain a fully developed embryo plus food supply. Anybody surprised by it ???



Not all seeds are that easy. Often it helps to free the embryo form the seed coat. Orchid seeds do not germinate readily. A single seed capsule contains 1,500 to 3,000,000 small seeds. Sowing the seeds *in vitro* makes it possible to germinate immature seed of green pods & mature = dry seeds



germinated orchid seeds = protokorms



seedling after 5 months growth in test tube
next:transfer to substrat of fern fibers: 7 months

1. Soak immature (green) seed capsule in 100% bleach solution for 30 minutes.
2. Dip the capsule in isopropyl alcohol or ethanol for 5-10 seconds. Remove . . .   capsule from the alcohol and carefully flame off excess alcohol.
3. Under aseptic conditions with sterile scalpel open capsule & scrape out seeds
4. Carefully layer the seed over the surface of the culture medium. Seal vessels.

Can plants make embryos from any piece of tissue ?

The generation of embryos in higher animals is a strictly sexual process In plants, however, embryos can be made from normal body cells too. Such **embryoids** are generated naturally in plant with viviparous embryos at their leaf, stem & root surface:

Red Mangrove (*Rhizophora mangle*)

Kalanchoe daigremontana, Tolmiea menziesii, Asplenium walking fern, bulbils of Dioscorea, the deadly seedlings of Rhizophora – the mangroves

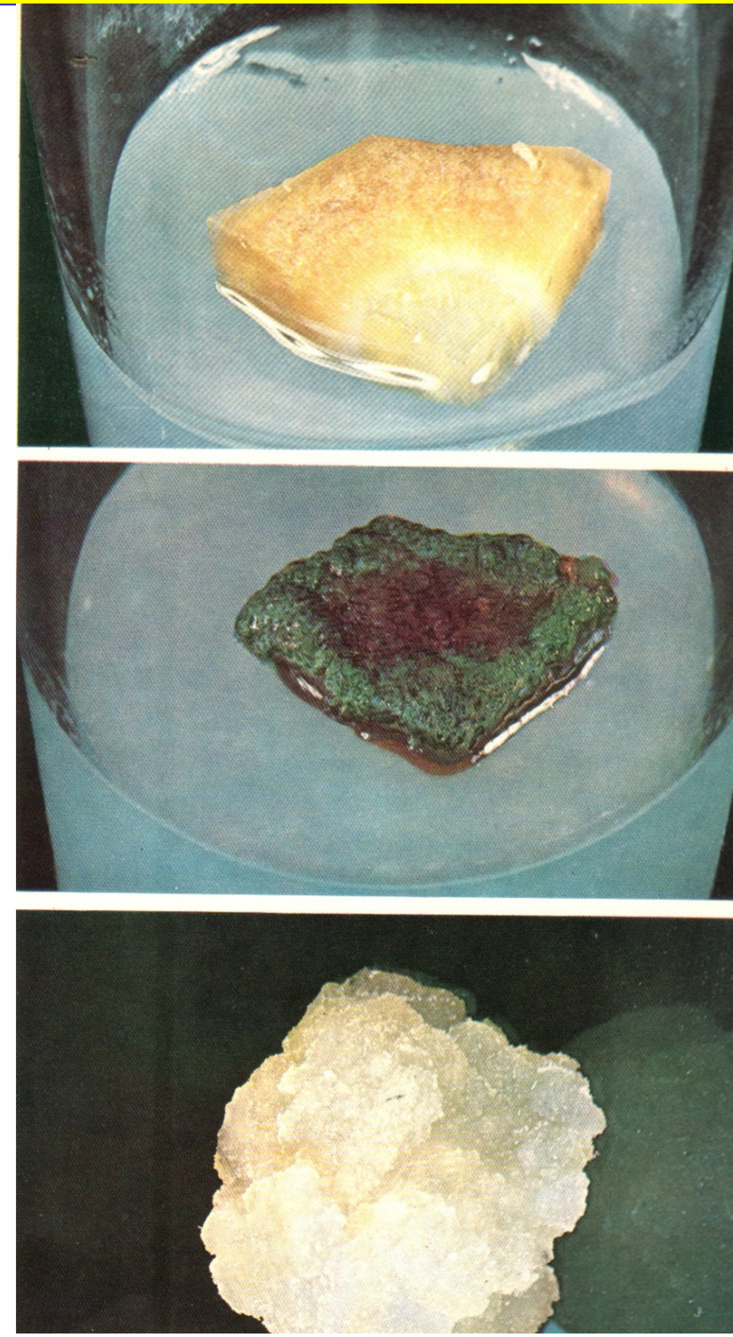
Can we grow a plant from any plant piece?

If we take a piece of carrot root & transplant it on an aseptic agar surface it will lose its root specialization & **dedifferentiate into a callus** that can even include green photosynthetic cells

The growing pieces are transferred onto new plates & develop a callus when supplied with sufficient nutrients & **hormones.**

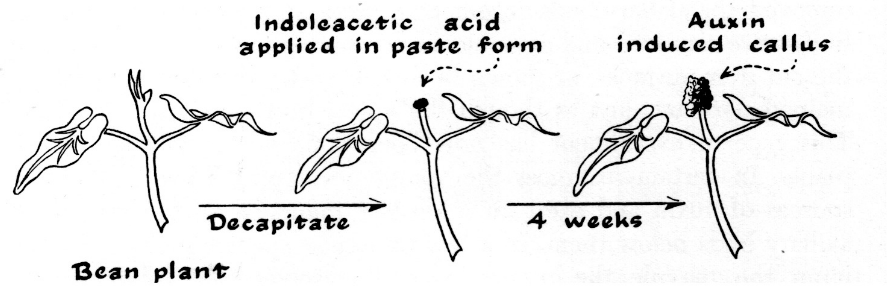
A surprising discovery was that you did not need the hormones to cultivate a callus from a natural tumor of the crown-gall disease. This is genetically transformed by the inducing *Agrobacterium tumefaciens* to make its own hormones and continuous callus growth.

One can induce callus also by giving excessive amounts of auxin in lanolin paste to a cut apex of *Phaseolus vulgaris.*

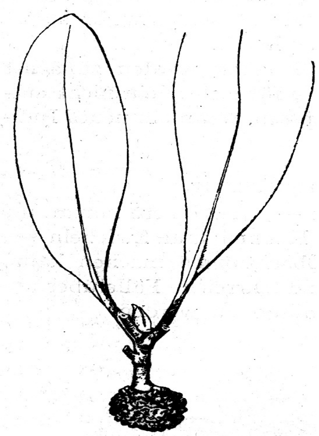


Is callus a confined to growing tissue on agar?

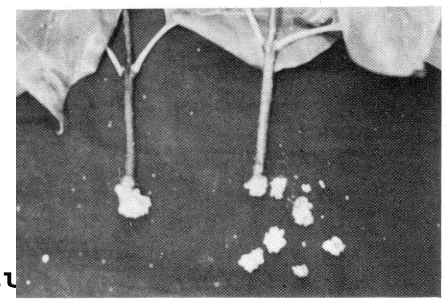
Callus is made by stems of bean leaders when supplied with exogenous auxin



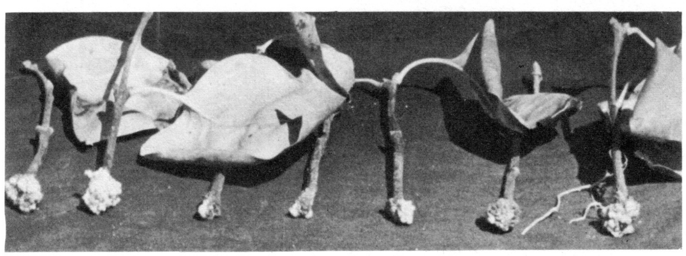
Application of auxin to decapitated leader of bean plants makes callus



Rhododendron arboretu
leaf-bud cutting
makes callus in wet
sand after 1 months



this system of plant propagation is that some cut- ırge callus. Varieties even within the species are thers. A plant propagator does not like to see cause apparently roots are seldom formed when ıg the treatments recommended are: dipping the left) and paring off excess callus growth (right).



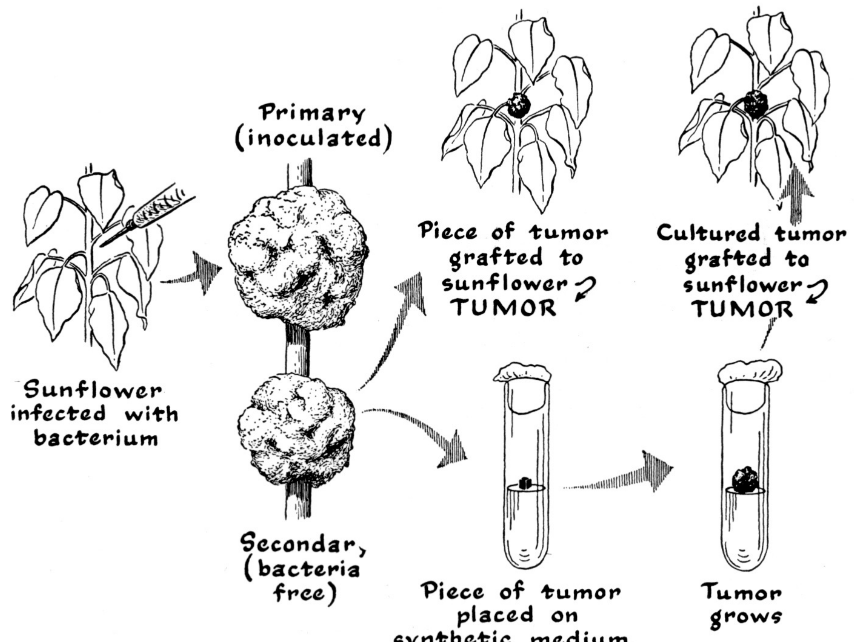
Here are the results. The two cuttings on the left show still more callus growth as a result of the powder treatment. Three in the middle are those that were pared and those at the right, one of which produced roots, received no treatment.

When stem cuttings are rooted by "constant mist" in a fog box some species show excessive growth of callus at the cut end.

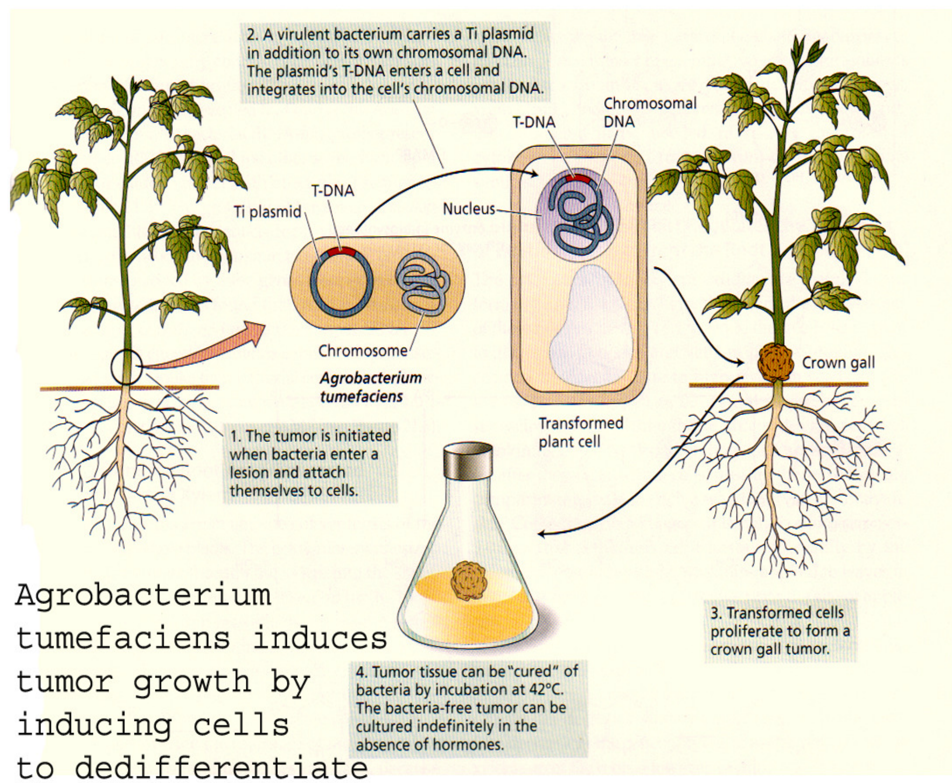
Dipping the cut into solution of root powder, we can overcome this and turn callus to grow roots.

Agrobacterium causes dedifferentiation of tissue ➔ Callus

A soil bacterium causes strange swellings around the root neck of plants, these cells are rapidly and uncoordinatedly growing like tumor cells, through a Ti plasmid getting incorporated into nuclear plant DNA. When such cells are transplanted into a aseptic culture **they propagate without the addition of hormones** (ideal choice for starters)



Tumor cells induced by crown-gall bacteri be easily grown in aseptic culture since require external supply of hromone.



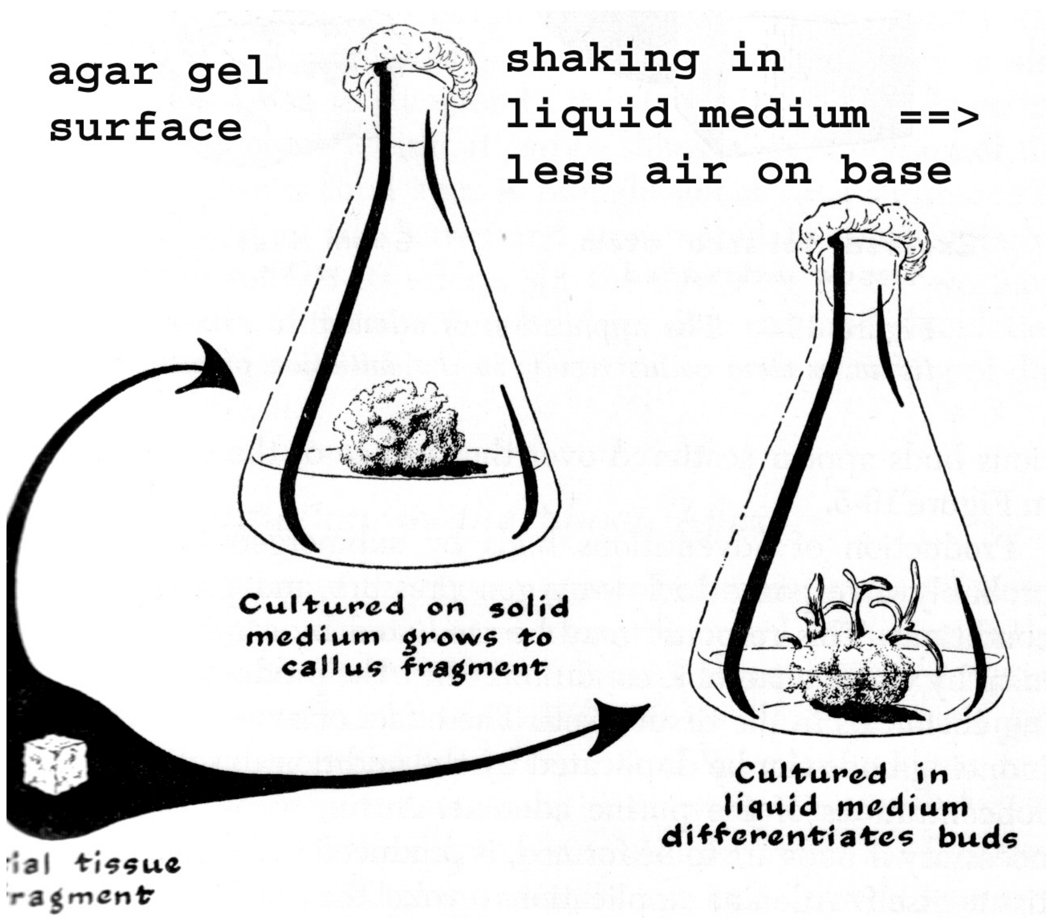
Agrobacterium tumefaciens induces tumor growth by inducing cells to dedifferentiate

Does the in-vitro technique and medium matter?

Callus can be made from various tissues: stem, root, leaf, fruit, even from pollen & is easily re-differentiated into shoots or roots.
Therefore **callus is the plant equivalent of animalistic "embryonic stem cells"**

Here you see callus growing preferentially on top of a agar surface, surrounded by lots of air ➔ callus

When half-way submersed in liquid medium without air supplied by vigorous shaking ➔ shoots



The formation of adventitious buds by excised tobacco callus tissue is controlled by conditions of aeration.

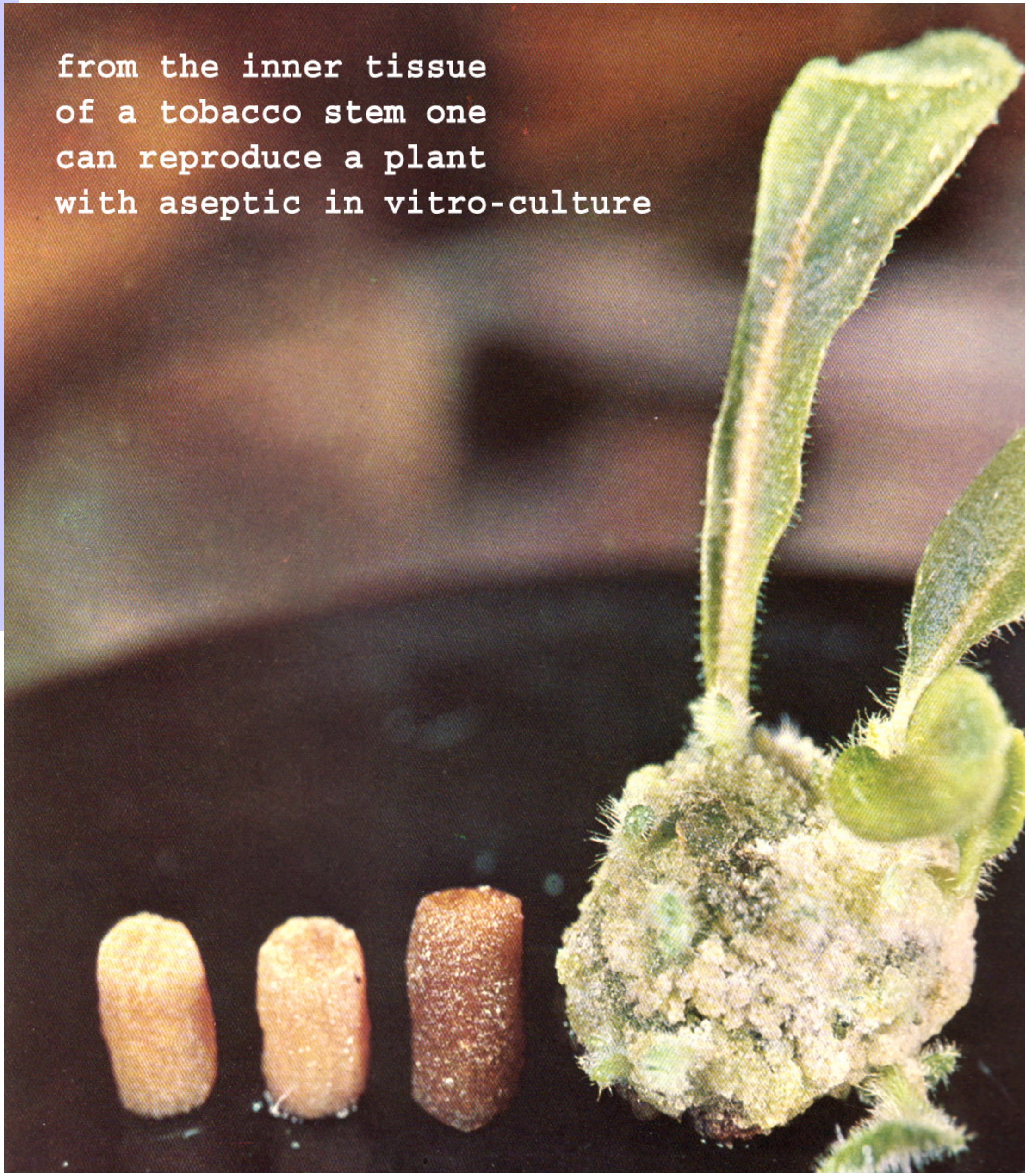
Can we regenerate a plant from any piece of tissue ?

Callus can be made from various tissues: stem, root, leaf, fruit, even from pollen showing that specialization of plant cells is easily reversed into what we could call **"growing parenchyma base tissue", callus or "embryonic stem cells"**

The major problem is to get the callus back to form differentiated cells & organs: for this you need to change the **hormone balance:**

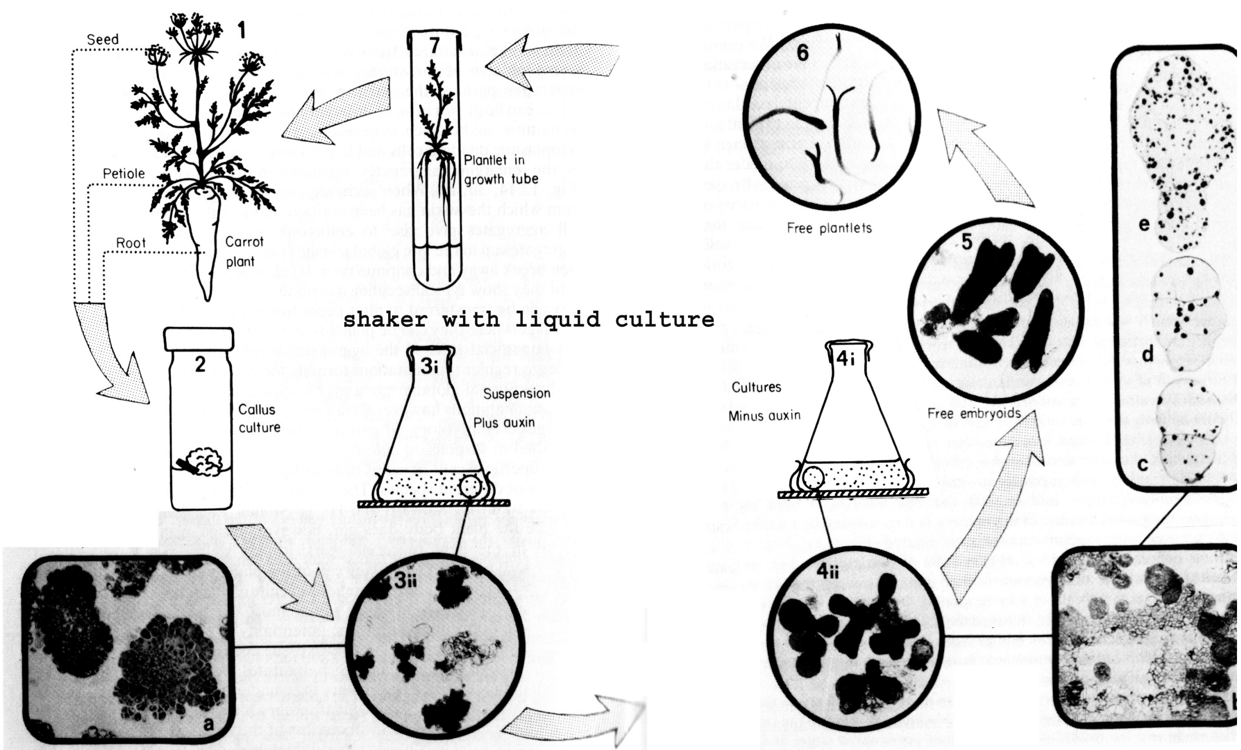
equal amounts of cytokinins & auxins ➔ callus

more cytokinins ➔ shoots



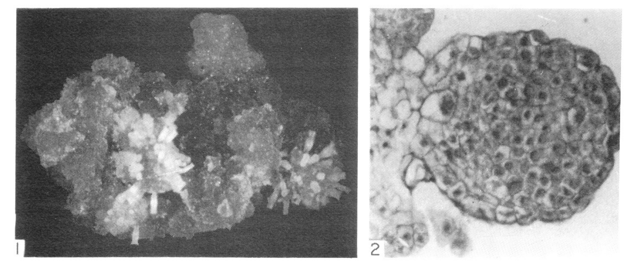
Can plants make embryos from any piece of tissue ?

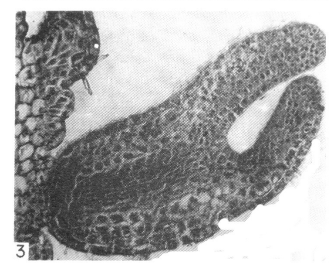
The generation of embryos in higher animals is a strictly sexual process
In plants, however, embryos can be made from normal body cells too.
Such **embryoids** are generated in the periphery of callus from Citrus macropcarpa, flower bud callus from Ranunculus scleratus (right picture suspension culture of carrots – the classical example 1972



Somatic generation of embryos in carrots.

1. petiole or root callus culture on agar 2. Suspension culture shaking to provide O
3. cell clusters or aggregates of small cells in suspension culture
4. when deprived of auxin embryoids develop at the periphery of the cell clusters (4ii)
5. globular stage (see right corner)grow into plantlets (6) tobe transfered to agar (7)





flower bud callus from Ranunculus sc with embryoids at the callus surface Left is a larger magnification that shows heart stage

Callus-derived seedlings of Ranunculus show lots of embryoids at the plant surface (induced vivipary

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