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 plants with viviparous embryos at their leaf, stem, and root surfaces:

- *Kalanchoe daigremontiana*
- *Tolmiea menziesii*
- *Asplenium walking fern*
- Bulbils of Dioscorea
- The deadly seedlings of Rhizophora – the mangroves

Vegetative Propagation

The amazing feature of plants (fungi, bacteria, lower animals) is 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 and plant organs from one plant part or tissue.

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

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

The Bud – an embryonal for shoot development

A **bud** is an embryonic shoot and occurs in the axil of a leaf or at the tip of the stem. A bud may remain dormant or form a shoot immediately.

Bud is protected by scales. When the bud develops, the scales drop off, leaving on the surface a bud scar. Since each year's growth ends in the formation of a bud, one can determine the age of any young branch.

Buds are classified according to:

- Location
- Status
- Morphology
- Function

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*
- **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)
- 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 --- bulbs

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*
- **Fleabane** *Erigeron*
- Saxifraga runners
- Potato tubers form on runner

### 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.

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 orchids (family orchidaceae) form above-ground storage organs called pseudobulbs, that superficially resemble 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.

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

Vegetative Propagation – Bulbil

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

Vegetative Propagation – viviparous leaves

Vivipary is the production of living seedlings instead of flowers. We find this in many plants from Walking fern, Tolmiea menziesii, Rhizophora, Kalanchoe, Poa all show somatic embryogenesis at the leaf margins. This can be improved by wounding the leaves in Kalanchoe & Begonia.

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.

Vegetative propagation is based naturally on multiplication of small corms on runners.
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
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
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.

Grafting to Root Stock

Hormone Auxin plus something else X
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.

Vegetative Propagation – Root Cuttings

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

Seeds – units of sexual propagation

Seed (kernel) is a small embryonic plant enclosed in a seed coat together with stored food supply. It derives from the ripened ovule of gymnosperm and angiosperm plants after fertilization.

Seeds do not appear like anything we are familiar with in the world of higher animals. The next best thing is perhaps the egg which is common among most invertebrates and vertebrates like reptiles and birds.

Seeds are the most powerful means of propagation of higher plants (embryophytes), much more so than the spores of lower plants. They are a means to attract and create food dependences of seed-distributing animals like the humans (see Michael Pollan: Botany of Desire).

Seeds can be small: *Begonia* 1 oz – 106 seeds or big: 1 coconut = 15 lbs.

All seeds have requirements to be met before they germinate. Most basic ones are humidity and agreeable temperatures. Hardy annual seeds like *Calif Poppies*, *larkspur* etc., germinate in the fall with seedlings that are able to

Stimulant of Seed Germination

Most seeds need **external clues** to germinate: for most seeds it is humidity or soil moisture together with temperatures above 10 C.

However there is one exception: the seeds of *Ismene* or Spider lily that germinate just when they are ready (**internal clues**). They germinate wherever they might be: in an ashtray a matchbox or in soil.
Seeds derive from the zygote & are encapsuled in fruits (derived from ovary). They are infinite marvels in size, shape, mode of distribution etc.

Seed Size

<table>
<thead>
<tr>
<th>Seed Source</th>
<th>Fruit/Seed Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coco-de-Mere</td>
<td>Aesculus glabra Cattleya fruit &amp; seeds</td>
</tr>
<tr>
<td>Seychelles nut</td>
<td>American chestnut, a tropical orchid</td>
</tr>
<tr>
<td>Catalpa pods</td>
<td>winged Catalpa seed, Paulownia capsule &amp; seeds</td>
</tr>
</tbody>
</table>

Embryo Size

Variability in size and shape find little interest with gardeners etc. who hardly ever take a look below the fruit/seed level.

Embryo Size - Dicots

<table>
<thead>
<tr>
<th>Species</th>
<th>Plant Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gingko biloba</td>
<td>[\text{Phytophthora}{\text{}}]</td>
</tr>
<tr>
<td>Phaseolus vulgaris</td>
<td>[\text{Apera}{\text{}}]</td>
</tr>
<tr>
<td>Avocado Persea amer.</td>
<td>[\text{Ricinus communis}{\text{}}]</td>
</tr>
<tr>
<td>Pea Pisum sativum</td>
<td>[\text{Beta vulgaris beet}{\text{}}]</td>
</tr>
<tr>
<td>Red Pepper Capsicum</td>
<td>[\text{Onion Allium}{\text{}}]</td>
</tr>
</tbody>
</table>

Embryo Size

Animal embryos at maturity, coconut embryo at maturity.
Monocots – extraction of embryo

Ze a mays  Triticum sativum wheat

requirements for germination for special seeds

Raising orchids from seeds is a gamble few gardeners win.
1903 French botanist Noel Bernard demonstrated that mycorhizal fungi help to provide sugar for the seed germination.
1924 Lewis Knudson (Cornell, US) germinated sterilized orchid seeds on a nutrient gel of agar + sucrose showing that fungus is not obligatory.

Seed s – requirements for germination: water, temp, air

All seeds have requirements to be met before they germinate. Most basic ones are humidity & agreeable temperatures. If you plant seeds, they require a porous and well-drained soil. If you “puddle the soil”, i.e. stirring it when wet – you remove air spaces + oxygen needed for germination.

Hardy annual seeds like California Poppies, larkspur Delphinium etc. are sown in late summer, germinate in the fall with seedlings able to survive winter.

Biennial seeds such as hollyhock Althaea, Bellis perennis, Campanula, Digitalis, Myosotis (forget-me-not), Verbascum mullein, Viola Pansy sown from June to August.

Seeds of hardy perennials are sown late April to early May. They will flower and make seeds at the end of September. Columbine Aquilegia, Bleeding hearts Coreopsis, Gas-plant Dictamnus, Christmas-rose Helleborus, Flax Linum, Lobelia but not hybrids like Phlox, Peones etc.

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.

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.
Polarity

Like magnets also plants have a clearly defined polar structure with the apical end producing leafy shoots and the basal end making roots.

If a moss spore is treated with colchicine or chloral hydrate it will not obtain polarity and cannot make the normal protonema threads but a bunch of unorganized callus cells

Bryopsis – a thread-like alga will make rhizoids depending on gravity

One can force the basal end to produce leafy shoots by adding a gypsum prop to apical end

The cell theory and its delayed experimental applications

Botanist Schleiden (1838) & Zool. Theodor Schwann (1939) formulated cell theory: all organisms are made of small universal units called cells. Cells are a characteristic feature of life as we know it.

Rudolf Virchow (1858): formulated principle of Totipotency of cells. All cells derive from cells only. Experiments to this question started with Botanist Vochting 1870s & continue to this day (stem cell research and ECMs = extracellular matrix proteins informing new cells about their role e.g. instead of scar tissue skin tissue)

Vochting’s dissection exp.: made increasingly smaller pieces of plant tissues while checking whether they maintained polarity. Distal portion of root or stem sections always produced leaves, proximal always roots. However, this was not response of cell but tissue; part organism

Vochting’s 2nd line: grafting & transplanting the transplanted scion is always determined to keep its polarity no matter what the host may have.

The birth of tissue culture – 1902

Bot. Gottlieb Haberlandt (1902): realized that one must make “attempt to cultivate vegetative cells of higher plants in suitable nutrients... The results of such experiments should also show the influences to which a cell is subjected in the multicellular organism or tissue.

Haberlandt started to grow cells and small groups of cells in micro chambers like hanging drops of various composition. He started what became tissue culture. **H. learned more than what he wanted**: (1) plant cells do not live in nutrient solution, xylem sap does not bring all the nutrients needed for live, (2) plant cells are surrounded by cell walls restricting access of larger molecules (3) animal cells have no walls and like erythrocytes etc. are easier to cultivate in natural fluids like lymph & blood! H. thought that by using green cells he could avoid these problems but photosynthetic cells are normally highly mature (lesson 2).

Haberlandt’ 2nd attempt (1913-1922): wound healing led him to propose the existence of “wound hormones” in plants. Hormones were ultimately the important tool that made isolated plant cells develop and grow.

The birth of animal & plant tissue culture – 1907

Harrison (1907): used same idea & methods as Haberlandt. Successfully cultivated neuroblasts of the frog in clotted lymph solution.

Burrows studied with Harrison and then joined the lab of Carrel: they established the standards of producing excised animal tissues in a nutrient consisting of blood plasma and embryo juice. However, this mixture is not exactly defined & much research had to be done to isolate the substances essential to grow animal cells (vitamins, growth factors, hormones, etc.). Much was learned but culture remained empirical art.

Kotte (1922, student of Haberlandt) succeeded cultivating root tips of pea and maize using Liebig’s meat extract or yeast. In US Robbins (1922) kept his roots alive for 22 weeks by subculturing them (dilute and change medium)

White (1939) succeeded cultivating tomato & clover roots for years plus undifferentiated tissue (callus culture) for more than a year.

Van Overbeek, Conklin and Blakeslee (Science 1941) used coconut milk (liquid endosperm and hence a kind of embryo juice) to successfully grow Datura embryos isolated from seeds.
In order to determine what a plant needs from the soil you must bring it on an inert medium that does not contribute anything to the root solution. Such is distilled water, vermiculite, washed sand, boiled peat moss etc. Experiments like this established that plants need some elements in macroscopic amounts: C, O, N, S, Mg, K, P, Ca, Fe & other elements in traces: B, Zn, Se, Cu, Mb, Cl … If one of the elements (for the water plant on the left - Cl) is in short supply, this will stop growth drastically even if all other elements are supplied (Liebig’s law of the minimum).

Organ culture started in 1922 with roots. What did we learn?

Kotte 1922 in Berlin, directly inspired by Haberlandt could keep roots alive in nutrient solution for some time Robbins 1922 in USA kept his root cultures even longer than Kotte by transferring to new, unused medium (good move!) but even that did not keep them alive for more than a few months. They needed something else ...

Robbins established that transfer of subcultures exhibits progressively diminishing growth rates Why are the dear roots not growing like in a normal plant? Let us add a bit of something!

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

Frits Went was of the opinion that little leaves grow better & larger when they have older brothers. In some plants one can observe, that first leaves are tiny compared to those arising later (Went & Thimann 1937) they accelerate their growth upon addition of extracts from older leaves or cotyledone or if purified of the purines: adenine & hypoxanthin

Adenine is a nucleobase & a purine derivative as in adenosine triphosphate (ATP) and the cofactors nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD), and protein synthesis, as a chemical component of DNA and RNA. 

This work has been completely forgotten. But I … work on pea leaf growth ….

What did we learn? … Roots need vitamins!

Robbins established that transfer of subcultures exhibits progressively diminishing growth rates, even after he added sucrose ….

James Bonner 1948 found out what that was isolated tomato root tips stop growing in normal mineral solutions unless they get something else:

Flax roots needed only thiamine, tomato roots thiamine & pyridoxine. pea & clover roots need thiamine plus nicotinic acid. These substances are water-soluble vitamins of the B-complex. Thiamin is vitamin B1, Nicotinic acid is vitamin B3, pyridoxine is B6, Vitamin B6 occurs only in minute amounts in most plants (except dragon fruits).
Let us grow … Stems… in culture. What do we learn?

C Darwin already concluded that some growth factor must account for the tropistic bending of stems. F.Went found that basal end of coleoptile segments releases a substance that will cause coleoptiles to grow & bend.

There are cheaper synthetic versions around that are also metabolically stable.

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

IAA is produced in cells in the stem apex (bud) and young leaves of a plant. Plant cells synthesize IAA from tryptophan. IAA induces cell elongation & cell division with important consequences for growth & development.

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

Auxin is produced in pollen & in the developing endosperm after pollination. Pollination induces ovule growth into a seed. The surrounding fruit growth often depends on auxin produced by developing seeds; => role of achenes in compound fruits of strawberry. When you remove the achenes (little kernels = seeds or fruits) the surrounding tissue of the receptacle fails to grow. Spraying de-seeded strawberries with auxin restores growth of the now seedless fruits.

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

The influence of light can also be observed in in-vitro culture: Here you see the previous tomato ovary being grown under light or darkness.

Note that the dark-grown tissue develops many roots (right) while light seems to be necessary for true fruit maturity with softening and lycopene formation (left).

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

Organ culture appears easy & natural since it allows to grow a growing embryo (= seed) & ovary (= fruit) to ripen to maturity along their normal and natural path of development.

However, certain hybrids stop their development a few steps after fertilization and hence we have here a method to grow the hybrid ovaries in culture without the restraints of species-specific nursing!
Let us grow .... Fruits ... without pollinators

Pollination not only induces (1) fruit growth but also (2) inhibits flower abscission, a very common process in many plants.

Auxin is produced in pollen & in the developing endosperm after pollination. Pollination induces ovule growth into a seed. The surrounding fruit growth often depends on auxin produced by developing seeds; => role of seeds in the berries of tomato (1952)

Spraying with auxin instead of pollination has the same effects:
1. Growth of a tomato berry that is ........................, however.
2. No abscission of flower.

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 from the seed coat. Orchid seeds do not germinate readily. A single seed capsule contains 1,500 to 1,000,000 small seeds. Sowing the seeds in vitro makes it possible to germinate immature seed of green pods & mature = dry seeds

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.

How do these organ-based growth factors interact?

Haberlandt & later Bonner started a most ambitious project: to figure out whether their knowledge of plants was sufficient to simulate their growth & development. This crucial test that modern research has also to meet...

The root growth factor thiamine is synthesized in the leaves, which hence control root growth in tomato plants.

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.

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.

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**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).

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**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.

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**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.
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 embroids are generated in the periphery of callus from Citrus macrocarpa, flower bud callus from Ranunculus scleratus (right picture: suspension culture of carrots – the classical example 1972.

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

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.

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

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.