PLANT PROPAGATION

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Summary

The preponderance of food and fiber for human consumption is derived from plants. The ability to domesticate crop plants was a pivotal point in human evolution. It permitted the transition from a predominantly nomadic lifestyle to one of more centralized communities of towns and villages. In turn, this allowed for stratification in the community for specialized activities not directly related to acquiring food. Several agricultural disciplines have evolved from the need to domesticate crops. These include disciplines for selection of crops for superior characteristics (plant breeding), multiplication of selected crops (plant propagation), cultivation of these crops (agronomy, horticulture, forestry, entomology, plant pathology, etc.), and processing and preserving harvested crops (food technology). This chapter will be a brief overview of plant propagation. It is not possible to provide a detailed description of all the techniques used for plant propagation (Table 1), but I will attempt to highlight some of the current emerging technologies with their potential for future crop production. The chapter will be divided into methods for sexual (seed) and asexual (vegetative) propagation.

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<th>Propagation method</th>
<th>Description</th>
<th>Commercial use</th>
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<tr>
<td>Seeds</td>
<td>Seeds form the sexual generation of the plant's life cycle. Seeds are either directly sown in the field to produce seedlings or sown under protected conditions.</td>
<td>Seed propagation is the most common form of propagation used to produce agronomic, horticultural, and forestry plants.</td>
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Table 1. Summary of Propagation methods used for Crop Production

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
<th>Examples</th>
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<tr>
<td>Cuttings</td>
<td>Cuttings are detached plant organs (stem, leaf or root) used for clonal propagation. Stem cuttings are the most common form of cutting propagation and require the regeneration of a new (adventitious) root system.</td>
<td>Cutting propagation is the most common form of commercial clonal (vegetative) propagation.</td>
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<tr>
<td>Grafting and budding</td>
<td>Grafting is the joining of two or more genotypes in a way that they unite and form a single plant.</td>
<td>Grafting is the clonal propagation method of choice in cases where plants will not easily root from cuttings. It is also used in cases where there is a distinct advantage to using a special understock (such as dwarfing or disease resistance).</td>
</tr>
<tr>
<td>Micropropagation</td>
<td>Micropropagation is the formation of new plantlets in tissue culture.</td>
<td>Micropropagation is used to mass propagate high-value crops that are slow to multiply by other clonal propagation methods. It is also used to produce disease-free stock plants.</td>
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<tr>
<td>Division</td>
<td>Division is the separation of a single plant into multiple pieces each containing a portion of the growing point and root system.</td>
<td>Many plants naturally multiply by division. It is an inexpensive propagation method for perennial species that form crowns or modified stems like many geophytes (bulb crops).</td>
</tr>
<tr>
<td>Layering</td>
<td>A layer is analogous to a stem cutting, but the stem forms roots while it is still attached to the mother plant. Plants that produce stolons or runners naturally propagate by layers.</td>
<td>Layering is a relatively inexpensive propagation method that requires no special equipment. It is not a major commercial practice except for mound layering (stooling) in apple and runners in strawberry.</td>
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1. Sexual Propagation

Among the many adaptations plants have made to cope with environmental stresses, the evolution of seeds is one of the most important. Most commercial food, oil, timber, fiber and ornamental bedding plant crops are propagated using seeds. Seeds are both the starting point and final product of our most important agricultural commodities (i.e. cereal and legume crops) and therefore, they are the foundation of our agricultural cropping systems. There are significant challenges for the seed industry to maintain and improve germination characteristics of seeds to meet the demand for food production predicted for an increasing world population. Emerging technologies for seed production and germination can be seen in the areas of testing, germination enhancement, storage, and germplasm preservation.

1.1. Seed testing

Seed producers use seed testing to evaluate seed quality during seed production, handling and storage, as well as to comply with international, federal or local seed laws.
High quality seeds are evaluated by tests for seed purity, viability, vigor and seed health. Of these, the areas of seed purity and seed vigor evaluation are being significantly impacted by emerging technologies.

Purity is the percentage by weight of the “pure seed” present in a sample. Purity determination requires a trained seed analyst usually certified by a national or international agency. Seed purity is comprised of both a physical and a genetic component. During purity testing, seed lots must be evaluated for physical contaminants such as soil particles, plant debris, other inert material, and weed seeds. However, it is the genetic component of the evaluation process that is undergoing significant change.

![Figure 1. An example of seed vigor in greenhouse grown pansy seedlings. Both flats show similar percentage emergence, but seedlings from the higher vigor seed lot in the upper panel are germinating quickly and more uniformly.](image)

For genetic purity, the seed analyst determines if the sample is the proper cultivar and identifies the percentage of seeds that are either other contaminating cultivars or inbreds
in a hybrid seed lot. The analyst has relied upon field evaluation, physical characteristics such as seed color, morphology, and various chemical tests to determine genetic purity. More recently, direct methods of genetic evaluation of seed lots have been employed including isozyme (characteristic seed proteins) separation by electrophoresis, and DNA fingerprinting. These methods have become increasingly relevant due to the need to evaluate seed lots for genetic modifications generated through genetic transformation (GMOs). Currently, the two most important genetic modifications in commercial crops are for insect resistance (i.e. the toxin from Bacillus thuringiensis) and herbicide resistance (i.e. gene for glyphosate tolerance), while cultivars with improved nutrition will become important in the future (e.g. golden rice). Testing has become important to ensure purity of the seed lot, but also to prevent unlicensed use of modified seeds and to certify that a seed lot is GMO-free where this might be important for use of the harvested crop.

Machine vision has the potential to make a significant impact on seed conditioning and purity evaluation. Machine vision utilizes a digital camera to capture images of seeds that are subsequently evaluated by computer for either surface seed characteristics or internal chemical makeup. This has the potential for speeding up evaluation of seeds for purity by reducing the time required for direct analyst evaluation of the seed lot. For example, physical characteristics of grass seeds have been utilized to separate tall fescue from ryegrass seeds using machine vision.

Internal characteristics of seeds can also be evaluated by machine vision by using cameras that evaluate in wavelengths other than the visual spectrum. For example, near-infrared spectroscopy can be used to evaluate a number of seed characteristics including seed moisture, oil composition, and contaminating fungi. Machine vision may also become an important aspect of evaluating seed lots for seed vigor. Seed vigor is the ability of a seed to produce usable seedlings under less than optimal environments (Figure 1). Compared with standard germination tests that evaluate seed viability, seed vigor is a better predictor of field emergence. Vigor also declines in stored seeds prior to any noticeable loss in viability. This makes measures of seed vigor a good predictor of imminent loss of viability in storage.

Figure 2. Evaluating seedling size for vigor determination. Digital image of impatiens seedlings on the left were detected by computer analysis and measured for length and area in the right panel.
The most common commercially used vigor tests are the cold test and accelerated aging test. These tests are relatively labor intensive and can be difficult to standardize between seed labs, but they have proven useful tests for seed producers. An alternative is to use seedling growth under controlled environments as an indicator of seed vigor. There are commercially available systems that analyze either digital images of emerged seedlings in a plug tray or seedling length (radicle) in controlled petri dish germination conditions (Figure 2). These are useful for small-seeded crops like ornamental bedding plants or vegetables that are difficult to test using standard cold or accelerated aging tests for vigor. However, as this technology improves and becomes mechanized, machine vision may prove useful for testing a variety of crop seeds for viability and vigor.

1.2. Treatments to enhance seed germination

There are a number of commercial seed treatments to enhance seed propagation either by facilitating mechanical sowing or improving the ability of the seed to germinate over a wide range of environments. Three worth highlighting are seed coatings, priming and pregermination.

Figure 3. Pelleted or film coated seeds. Pelleted seeds have a round shape (upper panel), while film coated seeds retain the original shape of the seed (lower panel).
Seeds are coated to change the shape of the seed (pelleting) to allow precision mechanical sowing or covered with a thin polymer film (film coating) that acts as a carrier for seed protectants (Figure 3). The technology to coat seeds comes from pill manufacturing in the pharmaceutical industry. Pelleting builds inert material around the seed to make it a uniform, round shape. Many small-seeded ornamental bedding plant and vegetable crops are pelleted for precise mechanical sowing. In contrast, film coating adds only 1 to 5% to the weight of a seed compared to over 1000% for pelletized seed. Even so, this can still aid in precision sowing by improving flowability. Fungicides and beneficial microbes can be added to both pellets and film coatings and is the major benefit to film coating. Although chemical treatments (i.e. fungicides) dominate industry seed treatments, the novel use of treating seeds with beneficial microbes presents an interesting alternative to chemical treatments. Various biocontrol agents provide protection to seeds by the production of antibiotic substances; competition for space and nutrients; and parasitism. Common biocontrol agents include bacterial strains like *E. terrobacter, Pseudomonas, Serratia*, and fungal strains like *Gliocladium* and *Trichoderma*. Several studies show disease prevention with biologicals to be as effective as chemical treatment with fungicides. In addition, seed coatings can include nitrogen-fixing bacteria or seed safers to guard against herbicide injury during seedling emergence.

Seed priming is a controlled seed hydration treatment that can reduce the time it takes for seedlings to emerge (Figure 4). Seeds are hydrated under conditions that allow physiological processes associated with germination to proceed, but do not allow the radicle to emerge from the seed coat. After hydrating seeds for an extended time, seeds are dried back to near their original dry weight. These seeds can be handled as normal raw seeds or pelletized prior to sowing. Techniques used to prime seeds include osmotic, matric, and drum priming.

![Figure 4. Seed germination in primed versus non-primed seeds. Both groups of seeds germinate at about the same percentage (95%), but the speed and uniformity of germination is superior for the primed seeds.](image-url)
Osmotic priming is currently the most common technique used to prime seeds. It involves suspending seeds in an aerated osmotic solution (either salts or polyethylene glycol). Concentration of the osmotic solution determines hydration of the seeds. Matrix priming takes advantage of the water relations properties (matric potential) of various moist, solid carriers (like vermiculite or calcined clay) to partially hydrate seeds. It is most often practiced with large seeded crops such as corn.

The newer of these techniques to prime seeds is drum priming. This is a patented practice where seeds are tumbled in a rotating drum that is mounted on a weighing balance. As the drum rotates, water is sprayed uniformly on the seeds until a predetermined hydration weight is obtained. This is often a preferred method because it avoids having waste (salts of polyethylene glycol) to dispose of after priming is complete. In all cases, seeds are held in a controlled hydrated state for several days usually at cool temperature (~15°C). However, parameters for optimum seed priming (hydration level, time in controlled hydrated state, and temperature) are not the same for all seeds and needs to be determined for each species being primed.

Primed seeds will usually show higher seed vigor compared to raw seeds. This is especially noticeable when primed seeds are germinated under less than favorable germination environments. For example, it is common to prime lettuce seeds to overcome germination problems associated with high summer field temperatures (thermodormancy). Growth substances or biologicals (termed biopriming) can also be included in the priming solution for added seed enhancement.

In contrast to seed priming, pregermination allows germination to proceed to the point where the emerging radicle cracks the seed coat or the radicle is allowed to emerge a few mm beyond the seed coat. Germinated seeds are separated from non-germinated seeds and then seeds are dried slowly to near their original dry weight. Pregereminated seeds were introduced commercially in 1995 for bedding plant species (Impatiens) and is currently available for only a few high value ornamental crops.

The advantages of using pregerminated seeds include: production of 95% or better usable seedlings; fast, uniform germination; and because the seeds are dry, mechanical seeders can be used to sow seeds. The fact that these seeds are dry is a major improvement over older technologies to sow hydrated seeds with the radicle exposed in a protective gel termed fluid drilling. Fluid drilling was not a viable sowing technique because it was extremely time sensitive and required specialized sowing equipment. Dry pregerminated seeds have a shelf life of weeks and use current mechanical seeders.

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Bibliography

General reference

Seed propagation
Churchill D.B., Cooper T.M. and Carone R.A. (1993). Separation of mixed lots of tall fescue and ryegrass seed using machine vision. Transactions American Society Agricultural Engineers 35:1383-1386. [Demonstrates the ability of machine vision to automatically separate seeds with similar physical characteristics]
Contribution No. 32. Association of Official Seed Analysts. [Describes vigor tests that are currently being used by seed labs]

Apomixis
**Cutting Propagation**


**Grafting Propagation**

Errea P. (1998). Implications of phenolic compounds in graft-incompatibility in fruit tree species. Scientia Horticulturae 74:195-205. [A review article that discusses the evidence for phenolic compounds being associated with compatible grafts]


Honma S. (1977). Grafting eggplants. Scientia Horticulturae 7:207-211. [An example of grafted vegetable propagation to avoid soil-borne disease pests]


Santamour F.S. Jr. (1989). Cambial peroxidase enzymes related to graft incompatibility in red maple. Journal of Environmental Horticulture 7:8-14. [This is one of several articles by this author relating peroxidase isozymes with compatible grafts]

**Micropropagation**


Paul H., Kaigny G. and Sangwan-Norreel B.S. (2000). Cryopreservation of apple (Malus x domestica Borkh.) shoot tips following encapsulation-dehydration or encapsulation-vitrification. Plant Cell Reports 19:768-774. [This article serves as a good example of the technology being proposed for ultra low temperature germplasm storage of vegetative tissue]


**Automation and Robotics**


