Cycles of seed evolution

The Basics.

Sunlight plays a much larger role in our sustenance than we may expect: all the food we eat and all the fossil fuel we use is a product of photosynthesis, which is the process that converts energy in sunlight to chemical forms of energy that can be used by biological systems. Photosynthesis is carried out by many different organisms, ranging from plants to bacteria (Figure 1). The best known form of photosynthesis is the one carried out by higher plants and algae, as well as by cyanobacteria and their relatives, which are responsible for a major part of photosynthesis in oceans. All these organisms convert CO2 (carbon dioxide) to organic material by reducing this gas to carbohydrates in a rather complex set of reactions. Electrons for this reduction reaction ultimately come from water, which is then converted to oxygen and protons. Energy for this process is provided by light, which is absorbed by pigments (primarily chlorophylls and carotenoids). Chlorophylls absorb blue and red light and carotenoids absorb blue-green light (Figure 2), but green and yellow light are not effectively absorbed by photosynthetic pigments in plants; therefore, light of these colors is either reflected by leaves or passes through the Other photosynthetic organisms, such as cyanobacteria (formerly known as blue-green algae) and red algae, have additional pigments called phycobilins that are red or blue and that absorb the colors of visible light that are not effectively absorbed by chlorophyll and carotenoids. Yet other organisms, such as the purple and green bacteria (which, by the way, look fairly brown under many growth conditions), contain bacteriochlorophyll that absorbs in the infrared, in addition to in the blue part of the spectrum. These bacteria do not evolve oxygen, but perform

photosynthesis under anaerobic (oxygen-less) conditions. These bacteria efficiently use infrared light for photosynthesis. Infrared is light with wavelengths above 700 nm that cannot be seen by the human eye; some bacterial species can use infrared light with wavelengths of up to 1000 nm. However, most pigments are not very effective in absorbing ultraviolet light (<400 nm), which also cannot be seen by the human eye. Light with wavelengths below 330 nm becomes increasingly damaging to cells, but virtually all light at these short wavelengths is filtered out by the atmosphere (most prominently the ozone layer) before reaching the earth. Even though most plants are capable of producing compounds that absorb ultraviolet light, an increased exposure to light around 300 nm has detrimental effects on plant productivity.

Reaction Centers and Antennae.

Photosynthetic pigments come in a huge variety: there are many different types of (bacterio)chlorophyll, carotenoids, and phycobilins, differing from each other in their precise chemical structure. Pigments generally are bound to proteins, which provide the pigment molecules with the appropriate orientation and positioning with respect to each other. Light energy is absorbed by individual pigments, but is not used immediately by these pigments for energy conversion. Instead, the light energy is transferred to chlorophylls that are in a special protein environment where the actual energy conversion event occurs: the light energy is used to transfer an electron to a neighboring pigment. Pigments and protein involved with this actual primary electron transfer event together are called the reaction center. A large number of pigment molecules (100-5000), collectively

referred to as antenna, “ harvest” light and transfer the light energy to the same reaction center. The purpose is to maintain a high rate of electron transfer in the reaction center, even at lower light intensities.

Many antenna pigments transfer their light energy to a single reaction center by having this energy “ hop” to another antenna pigment, and yet to another, etc., until the energy is “ trapped” in the reaction center. Each step of this energy transfer must be very efficient to avoid a large loss in the overall transfer process, and the association of the various pigments with proteins ensures that transfer efficiencies are high by having appropriate pigments close to each other, and by having an appropriate molecular geometry of the pigments with respect to each other. An exception to the rule of protein-bound pigments are green bacteria with very large antenna systems: a large part of these antenna systems consists of a “ bag” (named chlorosome) of up to several thousand bacteriochlorophyll molecules that interact with each other and that are not in direct contact with protein.

In many systems the size of the photosynthetic antenna is flexible, and photosynthetic organisms growing at low light (in the shade, for example) generally will have a larger number of antenna pigments per reaction center than those growing at higher light intensity. However, at high light intensities (for example, in full sunlight) the amount of light that is absorbed by plants exceeds the capacity of electron transfer initiated by reaction centers. Plants have developed means to convert some of the absorbed light energy to heat rather than to use the absorbed light necessarily for photosynthesis. However, in particular the first part of photosynthetic electron transfer in plants is rather sensitive to overly high rates of electron transfer, and part of

the photosynthetic electron transport chain may be shut down when the light intensity is too high; this phenomenon is known as photoinhibition.

Photosynthetic Electron Transfer.

The initial electron transfer (charge separation) reaction in the photosynthetic reaction center sets into motion a long series of redox (reduction-oxidation) reactions, passing the electron along a chain of cofactors and filling up the “ electron hole” on the chlorophyll, much like in a bucket brigade. All photosynthetic organisms that produce oxygen have two types of reaction centers, named photosystem II and photosystem I (PS II and PS I, for short), both of which are pigment/protein complexes that are located in specialized membranes called thylakoids. In eukaryotes (plants and algae), these thylakoids are located in chloroplasts (organelles in plant cells) and often are found in membrane stacks (grana) (Figures 3 and 4). Prokaryotes (bacteria) do not have chloroplasts or other organelles, and photosynthetic pigment-protein complexes either are in the membrane around the cytoplasm or in invaginations thereof (as is found, for example, in purple bacteria), or are in thylakoid membranes that form much more complex structures within the cell (as is the case for most cyanobacteria) (Figure 5). leaves. This is why plants are green.

All chlorophyll in oxygenic organisms is located in thylakoids, and is associated with PS II, PS I, or with antenna proteins feeding energy into these photosystems. PS II is the complex where water splitting and oxygen evolution occurs. Upon oxidation of the reaction center chlorophyll in PS II, an electron is pulled from a nearby amino acid (tyrosine) which is part of the

surrounding protein, which in turn gets an electron from the water-splitting complex. From the PS II reaction center, electrons flow to free electron carrying molecules (plastoquinone) in the thylakoid membrane, and from there to another membrane-protein complex, the cytochrome b6f complex. The other photosystem, PS I, also catalyzes light-induced charge separation in a fashion basically similar to PS II: light is harvested by an antenna, and light energy is transferred to a reaction center chlorophyll, where light-induced charge separation is initiated. However, in PS I electrons are transfered eventually to NADP (nicotinamid adenosine dinucleotide phosphate), the reduced form of which can be used for carbon fixation. The oxidized reaction center chlorophyll eventually receives another electron from the cytochrome b6f complex. Therefore, electron transfer through PS II and PS I results in water oxidation (producing oxygen) and NADP reduction, with the energy for this process provided by light (2 quanta for each electron transported through the whole chain). A schematic Carbon Fixation.

Electron flow from water to NADP requires light and is coupled to generation of a proton gradient across the thylakoid membrane. This proton gradient is used for synthesis of ATP (adenosine triphosphate), a high-energy molecule. ATP and reduced NADP that resulted from the light reactions are used for CO2 fixation in a process that is independent of light. CO2 fixation involves a number of reactions that is referred to as the Calvin-Benson cycle. The initial CO2 fixation reaction involves the enzyme ribulose-1, 5-bisphosphate carboxylase/oxygenase (RuBisCO), which can react with either oxygen (leading to a process named photorespiration and not resulting in carbon fixation) or with CO2. The probability with which RuBisCO reacts with oxygen

vs. with CO2 depends on the relative concentrations of the two compounds at the site of the reaction. In all organisms CO2 is by far the preferred substrate, but as the CO2 concentration is very much lower than the oxygen concentration, photorespiration does occur at significant levels. To boost the local CO2 concentration and to minimize the oxygen tension, some plants (referred to as C4 plants) have set aside some cells within a leaf (named bundle-sheath cells) to be involved primarily in CO2 fixation, and others (named mesophyll cells) to specialize in the light reactions: ATP, CO2 and reduced NADP in mesophyll cells is used for synthesis of 4-carbon organic acids (such as malate), which are transported to bundle sheath cells. Here the organic acids are converted releasing CO2 and reduced NADP, which are used for carbon fixation. The resulting 3-carbon acid is returned to the mesophyll cells. The bundle sheath cells generally do not have PS II activity, in order to minimize the local oxygen concentration. However, they retain PS I, presumably to aid in ATP synthesis. Even though C4 plants have reduced amounts of photorespiration, the amount of ATP they need per amount of CO2 fixed is a little higher than in other plants, and therefore their total production rate is similar to that of plants with higher rates of photorespiration.

Some plants living in desert climates, such as cacti, keep their stomates closed during the day to minimize evaporation (stomates are openings in the leaf surface to enhance gas exchange). These plants take up CO2 during the night when the stomates are open, and temporarily bind the CO2 to organic acids in the leaf. During the day the CO2 is released from the acids and used

for photosynthesis. Plants using this mechanism of CO2 fixation are called CAM (Crassulacean Acid Metabolism) plants (Figure 7).

overview of these processes is provided in Figure 6.

Increasing CO2 levels.

The amount of overall CO2 fixation in plants growing under optimal conditions is limited primarily by the amount of CO2 available. Therefore, the increase of CO2 in the atmosphere will lead to somewhat higher rates of plant growth in environments where the CO2 concentration limits growth rates. This is usually the case in an agricultural setting, where nutrients and water availability are not limiting. However, also in natural conditions, where limitations other than the CO2 concentration will generally limit plant productivity, plant productivity has been found to often increase upon increasing the CO2 concentration.

Photosynthesis and respiration.

Virtually all oxygen in the atmosphere is thought to have been generated through the process of photosynthesis. Obviously, all respiring organisms (including plants) utilize this oxygen and produce CO2. Thus, photosynthesis and respiration are interlinked, with each process depending on the products of the other. The global amount of photosynthesis is on the order of a trillion kg of dry organic matter produced per day, and respiratory processes convert about the same amount of organic matter to CO2. A large part (probably the majority) of photosynthetic productivity occurs in open oceans, mostly by oxygenic prokaryotes. Without photosynthesis, the oxygen in the

atmosphere would be depleted within several thousand years. It should be emphasized that plants respire just like any other higher organism, and that during the day this respiration is masked by a higher rate of photosynthesis.

Diversity of Photosynthetic Organisms.

Even though plants are the most visible representatives of photosynthetic organisms, it should be emphasized that many other types of photosynthetic organisms exist. All photosynthetic bacteria other than the cyanobacteria and their relatives use only one photosystem, and for thermodynamic reasons they cannot use water as the ultimate electron donor. Instead, they can use reduced compounds such as H2S as donor. However, CO2 fixation occurs in these organisms. Some of these photosynthetic bacteria appear to have retained an evolutionary ancient arrangement of their photosynthetic apparatus, and are of interest for the analysis of evolutionary relationships of photosynthetic systems.

An extensive group of these photosynthetic bacteria, the heliobacteria, was discovered rather recently in the 1980s. The first representative of this group was isolated by the group of Dr. Howard Gest from a soil sample collected on the campus of Indiana University, and this isolation was the result of a fortunate coincidence of serendipitous events. Analysis of the heliobacterial reaction center has helped to lay the basis for the current concept that all photosynthetic reaction centers from the large variety of photosynthetic organisms are related to each other. The majority of bacteria cannot be maintained in pure culture (that is, without other oganisms). This has essentially limited analysis of photosynthetic prokaryotes to the relatively

small group of organisms that can be grown in pure culture. It is likely that the actual diversity of photosynthetic organisms is much larger than is known thus far. Indeed, species with novel photosynthetic properties are reported virtually every year. For example, recently an organism was reported that has chlorophyll d (a chlorophyll that is very rare in nature) as the main pigment. Moreover, several years ago, previously undetected and very small chlorophyll a/b-containing prokaryotes were recognized to be the major contributors to photosynthetic production in the open ocean. This emphasizes that much relating to biodiversity and photosynthesis is still to be discovered, and that these discoveries are not limited to tropical rainforests and other ecological settings of large popular interest.

Evolution.

In eukaryotes, photosynthesis takes place in the chloroplast, which has long been known to have prokaryotic features. Chloroplasts are thought to have evolved from a cyanobacterium (or close relative) that was in a symbiotic relationship with a eukaryotic, non-photosynthetic cell and was engulfed inside this cell. The cyanobacterium and the eukaryotic cell presumably were in a mutually beneficial relationship (endosymbiosis), with the photosynthetic organism sharing some of its produced carbohydrates with the host, and the host providing the photosynthetic bacterium with other compounds. The prokaryote slowly gave up its independence as well as its cell wall, and some of its genetic information was transferred to the nucleus of its eukaryotic host. The resulting chloroplast maintains a small, prokaryote-like circular DNA of its own (DNA is material carrying genetic information); this DNA contains the genetic blueprint to make many of the

membrane proteins needed in the chloroplast, which apparently are not easily targeted to and/or transported into the chloroplast. Occasionally, photosynthetic organisms are found where the chloroplast has retained a little more of the original cyanobacterial features. For example, in algae such as Cyanophora paradoxa plastids (called cyanelles) are found that resemble cyanobacteria in their overall morphology as well as in the fact that they are surrounded by a cell wall.

Not all chloroplasts have resulted from a single endosymbiotic event, but apparently from multiple events that occurred independently. Chloroplasts from higher plants and many green algae probably all result from the same endosymbiotic event, whereas chloroplasts from red and brown algae and from diatoms are the result of one or more other events. The situation is even more complicated in cryptomonads, a type of algae, and chlorachniophytes, photosynthetic amoebae, which apparently are the result of an endosymbiotic event of a eukaryotic alga in a eukaryotic host. The nucleus of the endosymbiont has been mostly degraded, resulting in a chloroplast enveloped by four membranes.

Early Events.

Chlorophyll is used by all photosynthetic organisms as the link between excitation energy transfer and electron transfer. Of particular note is the rate with which these transfer reactions need to occur. As the lifetime of the excited state is only several nanoseconds (1 nanosecond (ns) is 10-9 s), after absorption of a quantum, energy transfer and charge separation in the reaction center must have occurred within this time period. Energy transfer

rates between pigments are very rapid, and charge separation in reaction centers occurs in 3-30 picoseconds (1 picosecond (ps) is 10-12 s). Subsequent electron transfer steps are significantly slower (200 ps – 20 ms) but, nonetheless, the electron transport chain is sufficiently fast that at least a significant part of the absorbed sunlight can be used for photosynthesis. However, in the presence of excess light, damage may occur, which may originate from the formation of chlorophyll in “ triplet state”. In a triplet state two electrons in the outer shell have identical rather than opposite spin orientation. This triplet chlorophyll readily reacts with oxygen, leading to the very reactive singlet oxygen, which can damage proteins. To counter this damaging reaction, carotenoids are usually present in close vicinity to chlorophylls. Many carotenoids efficiently “ quench” triplet states of chlorophyll, thus avoiding formation of singlet oxygen. Chlorophyll in its free form is very toxic in the light in the presence of oxygen, because a close interaction with carotenoids is not always available under such circumstances. Therefore, all chlorophyll in a cell in aerobic organisms is bound to proteins, generally with carotenoids bound to the same protein.

Structure Determinations.

Because of the strict requirements of positioning of pigments and electron transfer intermediates to allow efficient electron transfer and minimal damage, the structure of pigment-protein complexes involved in photosynthesis is critical. With the exception of specific antenna complexes (such as phycobilisomes in cyanobacteria and chlorosomes in green bacteria), pigment-binding proteins are usually hydrophobic membrane proteins. This initially hampered attempts to elucidate the structure of these

complexes, as membrane proteins do not readily form the well-ordered crystals that are needed for high-resolution X-ray diffraction studies. However, in the 1980s the first structure of a membrane protein complex, the photosynthetic reaction center from a purple bacterium, was determined at high resolution (about 3 A; in comparison, the distance between neighboring atoms in a molecule is about 1 A). Investigators from the Max Planck Institute in Martinsried (Germany) who were involved with this work, most notably Hartmut Michel and Johann Deisenhofer, received a Nobel Prize in chemistry for this research. Since then, the structure of various reaction centers and antenna complexes has been determined at resolutions between 2 and 4 A. Figure 8 presents the structure of the photosynthetic reaction center from the purple bacterium Rhodobacter sphaeroides.

Similarities Between Reaction Centers.

Surprisingly, structural comparison of reaction centers from different photosynthetic systems showed that these reaction centers are basically similar to each other in terms of their overall three-dimensional structure. The basic reaction center unit consists of a protein complex with 10 transmembrane helices originating from either two identical protein subunits or from two similar polypeptides of common evolutionary origin. Each of these polypeptides contribute five membrane-spanning helices, and bind 2-3 chlorophylls (or, in the case of anoxygenic bacteria, bacteriochlorophylls). The fourth membrane spans from each subunit are held together by two chlorophylls, which are the chlorophylls in the reaction center that can be oxidized (give up an electron) upon excitation. Directly associated with the reaction center are proteins that bind antenna pigments. In the case of PS I

and similarly organized reaction centers from green and heliobacteria, the antenna portion, with six transmembrane helices, is attached to the N-terminal end of the reaction center proteins.

Implications of Photosynthesis Studies.

Photosynthesis has been studied in significant detail and photosynthetic systems are used frequently for development and application of advanced technologies, because photosynthetic systems are fairly well understood, are complex, and often undergo rather unusual biochemical reactions. Some examples are provided below.

Rapid electron transfer reactions.

A major difficulty in measuring enzyme kinetics at relatively short time scales (less than 1 ms) is that “ traditional” enzyme reactions require a mixing of substrate and enzyme, which usually takes a relatively long time. Kinetic analysis of light-driven reactions such as photosynthetic electron transport have a great advantage in this respect: reactions can be triggered simply by a light pulse, which can be even shorter than 1 ps. Moreover, many of the components participating in electron transfer have different absorption spectra depending on whether they are in the oxidized or reduced form. Using laser spectroscopy methods or more standard optical spectroscopy, it is relatively simple to follow the electron around on a timescale between 1 ps and several ms. The primary charge separation occurs in several ps, and reactions become gradually slower as they involve components that are further away from the reaction center. Because of the fast speed of early reactions, the electron and the “ electron hole” are

physically separated rapidly by a large distance (the electron generally has traveled about 2 nm to the other side of the membrane within 1 ns after charge separation), so that back reactions (charge recombinations) are not favorable anymore. Unpaired electrons on reactants that are transiently formed during redox reactions involving transfer of a single electron in many instances can be detected using electron paramagnetic resonance (EPR) and derived techniques (including ENDOR, electron nuclear double resonance, and ESEEM, electron spin echo envelope modulation). Many of these techniques can be used to kinetically follow redox reactions, and provide detailed information regarding electron spin distributions etc. Therefore, photosynthetic membranes and reaction centers have a prominent place as experimental systems in biochemistry and biophysics.

Organic molecules mimicking reaction centers.

Reaction centers are essentially an assembly of cofactors, held in the appropriate position and orientation by the protein environment. Several groups have used the natural system as a model to design organic molecules where the equivalents of the different cofactors are linked together by covalent bonds of various lengths. The result is the creation of a number of sophisticated molecules that serve as “ artificial reaction centers”. The more advanced molecules consist of two chlorophyll-type molecules linked together (one serves as the electron donor, the other as the acceptor), with the electron-accepting molecule linked to two quinones, which serve as electron acceptors in the natural system. The electron donating chlorophyll analog is linked covalently to a carotenoid, which can donate an electron to the oxidized chlorophyll. Upon excitation of the chlorophyll, a charge

separation occurs resulting in an oxidized carotenoid and a reduced quinone. This charge-separated state is formed with high efficiency. An example of such a molecule is presented in Figure 9.

Such molecules can be introduced into liposomes (artificial membrane vesicles) in a specific orientation, and when these are excited by light, a charge separation will occur across the liposome membrane. This results in an electric potential or proton gradient across the liposome membrane, which may be used for a variety of purposes, including ATP synthesis (the latter requires introduction of the ATP synthesizing enzyme into the liposome membrane). The groups of Ana and Tom Moore and Devens Gust at Arizona State University are leaders in developments in this area.

Genetic modification and protein engineering.

Because of the ease of detailed functional analysis of reactions and their rates in photosynthetic systems, reaction center complexes are frequently used to determine the consequences of small alterations in the polypeptides on the functional characteristics of the cofactors. Changes at single amino acid residues in the reaction center complex are sufficient to introduce large changes in the properties of cofactors, which in turn leads to altered electron transfer rates and efficiencies. Single amino acid changes at specific sites in the protein are easily introduced by genetic modification techniques, and resulting functional changes can be studied. An elegant example of such an approach is the modification of the midpoint redox potential of the bacteriochlorophyll in the reaction center of purple bacteria. The midpoint redox potential is correlated with the ease with which an electron is given off

after excitation and is taken up by the oxidized bacteriochlorophyll. Jim Allen, JoAnn Williams and coworkers at Arizona State University found that creating or deleting hydrogen bonds between the protein and the bacteriochlorophyll changed the midpoint redox potential of this bacteriochlorophyll in a rather predictable manner. In this way, reaction center complexes can be built with different oxidizing strengths, and effects on reaction rates and ultimately the effectiveness of alternate electron donors can be determined. Mutational analysis of photosynthesis proteins is simple in several bacterial systems. The reasons why this is so in selected cyanobacteria and purple and green bacteria are that (1) foreign DNA is taken up by the cell spontaneously or is introduced easily by other means such as electroporation (“ electric shock”), (2) once the DNA is inside it is incorporated into the organism’s genome at one predictable and specific site by means of a process named homologous double recombination, and (3) the organism can be propagated without relying on photosynthesis, for example using an added carbohydrate source.

Genetic approaches involving directed mutagenesis as described above have proven to be very useful in studying photosynthetic electron transfer and will be of increasing relevance for the design of photosynthetic organisms for biotechnological uses (see below). By this method the function of a large number of genes has been probed, and the role of individual domains and residues has been determined. Genomic sequencing projects are very useful in this respect, and the complete DNA sequence of one photosynthetic organism is already known. From the DNA sequence, the potential of the organism can be determined. The entire 3, 573, 470 nucleotide-long genomic DNA sequence of the transformable cyanobacterium Synechocystis

6803 was determined by Satoshi Tabata and coworkers at the Kazusa DNA Research Institute in Japan. This organism is used by several researchers, including in the Vermaas group at Arizona State University, to elucidate the role of many proteins thought to be involved in photosynthetic or other physiological processes. Meanwhile, other groups are working on the genomic sequence of two purple bacteria. With the genomic sequence in hand, the role of specific genes can be found by amplifying the gene of interest by means of polymerase chain reaction, cloning it into a plasmid, replacing the gene by a selectable marker (i. e., a piece of DNA coding for a protein inactivating a particular antibiotic, thus conferring antibiotic resistance), and analyzing the functional characteristics of resulting mutants. A website, CyanoBase (http://www. kazusa. or. jp/cyano/cyano. orig. html), has been established to facilitate searching of the genomic sequence of Synechocystis 6803, and a related site, CyanoMutants (http://www. kazusa. or. jp/cyano/mutants/), has been developed that accommodates information regarding targeted mutations and their effects in this organism.

Genetic modification of higher plants.

Globally, improving plant productivity by genetic means has been an important goal for many years. Even though initially it was hoped that crop productivity could be boosted by “ improving” the photosynthesis process, it has become increasingly apparent that improved productivity will be best accomplished by genetic modification of crop plants to introduce insect or pathogen resistance, or to yield improved vitality under marginal conditions (for example, at high salinity, which has become a significant factor in many agricultural areas because of continued irrigation). A pertinent example is

the development of varieties of cotton and other crops that express a pro-toxin from a bacterium, Bacillus thuringiensis, that is converted to a toxin in the midgut of particular insects such as caterpillars but not in other life forms. This allows efficient biological control as long as the insects do not develop resistance to this compound.

Genetic modifications to intrinsically and significantly improve the photosynthesis process have not yet been successful. The reason for the apparent inability to “ improve” the photosynthesis process itself presumably is related to the fact that photosynthetic systems have evolved over a relatively long period of time, and that the selection factors have not changed significantly in recent history. This has led to the emergence of a very effective photosynthetic apparatus that is difficult to improve upon by simply changing some amino acid residues or by introducing or deleting some genes. If relatively simple changes could have significantly improved the photosynthesis process per se, Mother Nature would have already found these as natural mutations are rather frequent. However, it is possible that significant progress in this area can made in the future, if new design paradigms for enzyme function and specificity can be developed. For example, if protein structures (particularly the structure of the active site) can be better modeled and predicted, one should be able to further improve upon the RuBisCO specificity of CO2 vs. oxygen. It is also important in this respect to det

GERMINATION

Keep in mind that the ovule in the ovary is what becomes the seed. The integument of the ovule becomes the seed coat. Inside the integument of the ovule was the embryo sac. The antipodals and synergids senesce and disintegrate. The central cell united with one sperm cell to make endosperm…a nutritive tissue that accumulates starch, protein, and fats to provide for the growth of the embryo. The egg cell of the embryo sac united with the other sperm cell to make a zygote. The zygote grows and becomes a true embryo inside the integument.

When you have a Dormant Embryo, a Storage Tissue, and a Seed Coat, then you have a SEED. In some seeds, the endosperm is retained as the storage tissue. In other seeds the endosperm is more or less used up to put storage chemicals into the embryo itself (commonly in the cotyledons). Below

Seed Germination

The seeds are carried inside the fruit by animals called dispersers. The animal usually feeds on the fruit (ovary wall), but “ tosses” the seeds (or passes them through its digestive system). This assures that seeds end up far away from the “ mother” plant, where they can develop without competition from “ mom.”

When it comes time for a seed to sprout, the diagram below might assist you.

Some seeds sprout with just water and reasonably warm temperatures. This is true of most common garden plants. Wild species usually have some kind of deeper dormancy to avoid sprouting in late summer/fall when the seeds

are dispersed. This assures that tender seedlings are not frozen at a young age, but do not appear until warm weather arrives in springtime. Examples of mechanisms are indicated in the figure below.

Also shown above is a mechanism for seed germination in two species: Barley and lettuce.

The hormone signal in barley (Gibberellic acid) activates DNA in the aleurone cells. Transcription and translation of a gene for amylase results in the production of that enzyme inside the aleurone cells. This enzyme is shipped by ER into the Golgi, sorted and packaged into vesicles, and exported through the cell membrane by exocytosis. The amylase is thus dumped into the endosperm area. There the amylase breaks down starch into sugar which is transported to the embryo. The sugar fuels respiration in the embryo so it can grow. The radicle protrudes from the seed coat, and germination is accomplished.

A similar mechanism exists in lettuce (no aleurone and a dicot), but the activating chemical is a pigment called phytochrome. This chemical exists in two different forms: Pr and Pfr. How a lettuce seed responds depends on how much of each of these two forms is in each cell. Typical lettuce seed batches germinate 30-60% if placed in darkness because at least this many seeds have enough Pfr to stimulate germination. If, however, you put the lettuce seeds in red light (660 nm), the red light causes all the Pr to change into Pfr. Now 85-95% of the seeds can sprout because they all have an abundance of Pfr inside. On the other hand, if you put lettuce seeds in far-red (730 nm) light, the far-red light causes all the Pfr to change into Pr. In far-red light,

then, all the seeds have essentially no Pfr and so very few (0-5%) actually sprout. You carried out these experiments in lab, so you know about this already.

SEED PROPAGATION

Most plants reproduce more of their kind through production of seeds. This is SEXUAL REPRODUCTION and it involves the exchange of genetic material between two parent plants. Many ornamental plants do not come “ true” from seed. To increase the numbers of these plants, gardeners and horticulturists use ASEXUAL PROPAGATION. In asexual propagation, the new plants are genetically exact copies or clones of a single parent plant. The methods used in asexual propagation range from taking leaf cuttings of African violets to grafting apple cuttings onto root stocks.

KEY CONCEPTSI. Sexual Propagation of PlantsA. Propagation by seed 1. Purchasing seed 2. Collecting seed a. Harvesting seed b. Storing seed 3. Germination of seed . Scarification of seed a. Stratification of seed 4. Sowing seeds indoors . Growing media a. Containers b. Sowing seed c. Care of seedlings started indoors 5. Sowing seed directly into the garden B. Spores 1. Collecting 2. Germinating II. Asexual Propagation of PlantsIII. Plant Patents

SEXUAL PROPAGATION OF PLANTS

Sexual propagation of plants involves the exchange of genetic material between parents to produce a new generation. Sexual propagation offers the following advantages:

It is usually the only method of producing new varieties or cultivars.

It is often the cheapest and easiest method of producing large numbers of plants.

It can be a way to avoid certain diseases.

It may be the only way to propagate some species.

PROPAGATION BY SEED

Propagation by seed is the most commonly known method of producing new plants. Common annual and biennial vegetables and flowers are grown easily from seed. Perennials grown from seed may take more than one season to flower. Lawn grasses are commonly grown from seed by the home gardener.

PURCHASING SEED

Use seed that is guaranteed reliable for freshness and purity. Purchase seed that is packaged for the current year. Most seed companies provide information on expected percent of germination. This figure tells what percent of seed is expected to sprout or germinate. Generally, 65-80% of most fresh seed sown will germinate. Of those seeds that germinate, 60-75% will grow into satisfactory seedlings.

When choosing seed, be sure to select varieties or cultivars that meet your requirements for size, color and growth habit. Choose varieties that will mature before frost. This can be estimated by using the number of days to maturity (found on the seed packet or in the catalog) and comparing it to the days between probable planting date and the average frost date of the area.

When considering seed not packaged for the current year, remember that the germination rate of most seeds drops dramatically after the first year.

Seed catalogs are not only helpful in purchasing seed, but they are also excellent references for cultural information. Catalogs and seed packets may provide information on country of origin, bloom time, specific germination requirements, cultural requirements and disease resistance. Some seed packets indicate if seeds have been chemically treated to prevent disease. Read packets and catalogs thoroughly and follow their recommendations. The more information the gardener possesses, the greater the chance of growing plants successfully.

COLLECTING SEED

Seed saved by the home gardener will probably be the result of random pollination by insects or other natural mechanisms. Random pollination results in seeds that produce plants that may not be identical to the parent plant. The seeds of HYBRID cultivars should not be saved.

Some plants make excellent candidates for seed saving. Common self-pollinated, non-hybrid and purebred annual vegetable seeds that can be saved include lettuce, beans, peas, herbs and HEIRLOOM tomatoes.

Saving seed saves money. It allows the gardener to maintain varieties that are not sold commercially. Many avid seed savers belong to groups that exchange seed through networks. Some seed saver groups specialize in keeping heirloom varieties. Many heirloom varieties are the great-grandparent plants of modern cultivars.

It may be tempting to bring home seeds or plants seen on vacation in foreign countries. However, this is how many serious insect and disease pests are introduced. A nonnative plant may become a noxious weed. Follow all import regulations for horticultural materials. The USDA Animal and Plant Health Inspection Service can provide information to travelers.

HARVESTING SEED

It is important to save seed from healthy plants because some diseases can be carried in seeds. Commercially grown seed is protected from disease problems because it is produced under very strict conditions with frequent inspection.

Harvest seed just before fruit is fully ripe. For flowers with exposed seeds, place the seed stalk or flower head in a bag and store in a warm, dry location. Seed will fall into the bag when it is completely dry. The seed of pulpy fruits should be separated from the pulp, washed and thoroughly dried.

STORING SEED

Once seeds are completely dry, place them in airtight storage containers marked with name and date saved. Store seeds at 40 degrees F with low humidity. The refrigerator provides these conditions.

Seed of many plants can remain VIABLE for up to 5 years if properly stored. However, it is best to use home-harvested seed during the following growing season. Some species of plants produce seeds that are short-lived. These

seeds must germinate immediately after they ripen or they lose their viability. Delphinium, onion and parsley are examples.

Before planting, it is a good idea to check stored seed for its germination rate. Planting these seeds directly in the garden may be a waste of time and effort if germination rate is very low. To check germination rate, place some of the seeds between paper towels that are kept constantly moist and between 65 and 70 degrees F. Check the seeds daily for germination. If the germination rate is 70% or less, consider buying new seed.

GERMINATION OF SEED

When germinating seed it is helpful to remember that a seed is made up of three parts:

an outer protective coat

a food supply under the seed coat (the endosperm)

an embryo of a young plant

The protective coat prevents sprouting until ideal growing conditions exist. Bringing seeds out of dormancy involves manipulating conditions to hasten germination. Even with ideal conditions, some seeds are still very difficult to germinate.

There are several factors that affect germination. Water (moisture), light (or dark), oxygen and heat play a part in triggering germination. In addition to environmental factors, seed must be viable.

Water is essential in the first phase of germination. Water penetrates the seed coat and causes the endosperm to swell. The seed coat, softened by water, splits open as the endosperm swells. The water dissolves nutrients in the endosperm making them available to the embryo and growth begins.

The growing medium must be constantly moist, but not wet. Any dry period may cause death of the sprouting embryo.

Light can stimulate or inhibit a seed’s germination. This determines whether the seed should be sown on the surface of the growing medium or below the surface. Check the seed packet or catalog for light requirements.

Oxygen is required by the embryo to begin growing. The seed must respire to break down the food stored in the seed. This is one reason for using a light, well-aerated growing medium for starting seeds.

Every seed has an optimum temperature range for germination. Many seeds have a fairly wide temperature range for germination, but some are limited to a narrow range. The temperature range is usually given on the seed packet or in the catalog.

The temperatures required by many seeds are higher than those in most homes. The desired constant temperature can be achieved through heating cables placed under germination containers. Setting flats or pots on radiators, the furnace or on the refrigerator will provide bottom heat. However, these locations may be too hot and cause the soil to dry too quickly.

Once germination occurs, a different, usually lower, temperature may be required for optimal growth of the seedlings.

SCARIFICATION OF SEED

The coat of certain seed is extremely tough and must be penetrated by special means. Particularly hard seed may be scarified. Scarification involves breaking, scratching or softening the seed coat to allow moisture penetration.

Two methods of scarification commonly used by the home gardener are mechanical and hot water. Mechanical scarification involves breaking or weakening the seed coat with a file, sandpaper or hammer. Hot water scarification involves placing seeds in water that is 170 to 210 degrees F. After the water cools, seeds should continue to soak for 12 to 24 hours. Then they are planted. Specific instructions for scarification are usually mentioned on the seed packet or in the seed catalog.

STRATIFICATION OF SEED

Some seeds will not break their dormancy unless exposed to a period of low temperature and moist conditions. Stratification requirements are usually indicated by the seed supplier. They can also be found in references, such as Michael Dirr’s MANUAL OF WOODY LANDSCAPE PLANTS, Steven Still’s MANUAL OF HERBACEOUS ORNAMENTAL PLANTS and plant propagation texts.

Plants that typically require seed stratification include many trees and shrubs, and certain perennials.

This period of “ chilling” or stratification can be accomplished by placing seed in a moist, sterile (pasteurized) growing medium, such as a mix of equal parts clean sand and peat or sphagnum peat moss, in a disinfested container. Enclose the container in a tightly sealed plastic bag and place it in the non-freezer section of the refrigerator. Towards the end of the stratification period seeds may start to sprout. Handle sprouted seeds carefully when transplanting into pots.

Some seeds may require both scarification and stratification to germinate reliably.

SOWING SEEDS INDOORS

Sowing seed indoors is the easiest and cheapest method of producing vegetables, annual flowers and some perennial plants. Plants with extremely small seeds or those that need a long growing season make excellent candidates for starting indoors.

Very small seed is difficult to plant outdoors because of size. Plants that require a long growing season may not have enough time to reach maturity unless started indoors in winter or early spring.

Supplies needed for indoor seed sowing include the following: fluorescent or grow lights, disinfested containers with excellent drainage, pasteurized (sterile) seed-starting medium and a location with proper temperature and ventilation.

GROWING MEDIA

Choose a medium with a loose, uniform, fine texture. A pasteurized mixture that is 1/3 soil, 1/3 sand, vermiculite or perlite, and 1/3 peat moss has the qualities of a good seed-starting medium. Retail garden centers carry mixes labeled for seed starting. Whatever is selected, be sure it is pasteurized (sterile). Using pasteurized soil prevents damping-off, a fungal disease that kills young seedlings. Pasteurized soil also helps to avoid weeds, diseases and pests.

Seed-starting media are usually low in fertility. This means that a regular fertilization program is very important once seedlings emerge.

CONTAINERS

Any recycled containers are adequate for seed starting provided they are disinfested, have good drainage and are at least 2 inches deep. Other container options include compressed peat pellets, peat pots, paper pots, plastic cell packs and flats.

Peat and clay containers tend to dry more quickly than plastic containers because they are very porous.

SOWING SEED

The correct timing of seed sowing is an important factor in successful indoor seed starting. In winter months, overanxious gardeners may sow seeds too soon. Seedlings that are held indoors too long perform poorly once transplanted into the garden. Most seeds should be sown 4 to 12 weeks prior to transplanting into the garden. The time it takes for seedlings to be ready for transplanting outdoors will vary.

An ACCLIMATION period before placing seedlings directly into the permanent growing site must be included. However, readiness for outdoor planting will vary with how quickly germination occurs, the growth rate and weather conditions. Quality and quantity of light, temperature and nutrients affect the growth rate. Seed catalogs and packets provide information on days to germination and weeks needed to reach transplant size.

Fill the container to within 1/4 inch of the top of the container with moistened seed-starting medium. After sowing seeds, keep the medium moist, not wet. To keep the medium moist, you may place the container in a plastic bag just large enough for the container. Seal the bag. The plastic bag keeps moisture in, but allows air exchange. The plastic bag method should not require any further watering until germination. Provide proper light and temperature conditions.

Once seedlings germinate, remove the container from the plastic bag. Place the container in a location that has high light intensity and cooler temperatures.

Use the following rules to sow seed, depending upon seed size. Sow very small seeds by sprinkling on top of the medium and pressing in. Use a fine mist of water to gently wash seed into the growing medium. Sow medium-size and larger seed in rows 1 to 2 inches apart, and 1/8 to 1/4 inches deep. If no depth is specified on the seed packet, use the general rule of planting the seed at a depth twice the diameter of the seed.

When sowing in a tray or flat, sowing in rows is preferred over scattering seed. This method provides better air circulation than scattering of seed.

When seedlings are crowded, they may become tall and spindly. To avoid the need to transplant seedlings from a seed flat to pots, you may sow seeds directly into cell packs or peat pots. Plant two or three seeds per cell or pot. When they germinate, remove the two less vigorous seedlings.

CARE OF SEEDLINGS STARTED INDOORS

TRANSPLANTING

Once the seeds have germinated, you will see two seed leaves or cotyledons. Eventually, these seed leaves will wither. Next, true leaves will form. These true leave look like the plant’s typical leaves. Transplant the seedlings to individual containers once the first set of true leaves appear.

Use a pencil, small stick or other narrow tool to lift seedlings from the seed flat. Plant each seedling in its own small pot filled with pasteurized growing medium. The new container should be no larger than a 2-1/2- to 3-inch pot.

Hold the seedlings by a leaf between the thumb and forefinger. Handling by the leaf avoids damaging the fragile stem which would kill the seedling. If a small leaf is lost in transplanting, the plant can recover and grow normally. Expose seedlings’ roots to air as briefly as possible. Lightly firm the soil around the seedling.

Transplanting temporarily slows or stops the growth of seedlings. Sowing into cell packs bypasses the need to transplant the seedlings.

WATERING SEEDLINGS INDOORS

Watering can be a cause of seedling failure. Keep soil moist but not wet. Small, tender seedlings dry out rapidly and can die. Remember that roots always must have oxygen, as well as water; therefore, do not keep the soil soaked. Water when the surface of the soil begins to dry out.

Bottom watering helps prevent damage to the seedlings caused by a hard stream of water. Bottom watering also encourages deep root development and ensures that the entire depth of soil receives moisture. Do not let the pot or flat sit in water longer than it takes for all of the soil to become moist.

TEMPERATURE FOR SEEDLINGS INDOORS

Keep seedlings in a well-ventilated, cool location. The temperatures should be 55 to 60 degrees F at night and 65 to 70 degrees F during the day. These temperatures encourage compact, bushy, vigorous growth while minimizing disease.

LIGHT REQUIREMENTS FOR INDOOR SEEDLINGS

Seedlings require bright light immediately after germination. One warm-white, 40-watt bulb and one cool-white, 40-watt bulb used together are adequate for seed starting and seedling growth. Fluorescent lights can be used for one year before replacement is recommended.

Special grow lights are also suitable, but more expensive. The lights should be no more than 6 inches above the top of the seedlings. Mount the light fixture so it can be raised as the plants grow in height.

Day-length requirements vary with different plants. Petunia, snapdragon, phlox and China aster require short daylight periods of 10 to 12 hours. However, most plants that are started from seed benefit from 16 to 18 hours of light.

FERTILIZING SEEDLINGS INDOORS

Because the growing medium used to start seedlings is usually low in nutrients, a regular fertilization program is important for proper plant growth. Apply a liquid fertilizer high in phosphorous weekly. Fertilizer with a 1-2-1, N-P-K ratio is recommended. Dilute fertilizer 1/4 to 1/2 the label’s recommended strength and apply sparingly. Always use a liquid form of fertilizer.

PINCHING SEEDLINGS

Pinching the growing tips of seedlings will result in more branching. This produces a fuller, stockier plant.

HARDENING OFF SEEDLINGS BEFORE TRANSPLANTING INTO THE GARDEN

Plants grown indoors must be gradually introduced to outdoor conditions. If seedlings are not hardened off, leaves may be burned by the intensity of the sun or damaged by wind. Acclimate plants by first placing them in a cool, protected location, such as a porch or shaded COLD FRAME.

This first step in hardening off allows plants to adjust to outdoor temperatures. After 7 to 10 days, move seedlings into a shaded area of the garden for 2 to 3 days. This will prevent sunscald. Finally, hardened

seedlings can be planted directly into the garden as weather permits. Planting on a cloudy day or late in the evening is a sensible precaution.

TRANSPLANTING SEEDLINGS INTO THE GARDEN

The garden soil should be adequately dry to prevent compaction. At this point, plants will again experience transplant shock and a setback in growth. Plants must adjust to dramatically different nutrient levels, soil temperatures, moisture levels and soil tilth in the garden.

Pull apart the lower portion of the root mass to get the roots growing outward. If seedlings have been grown in peat pots, pull apart the bottom of the pot and roots. Although seedlings may be planted without removing the pot, be sure to maintain the same soil level. Trim away any of the pot that is above the soil line. The exposed portion of the peat pot acts as a wick and dries out the entire pot and roots.

Water seedlings into the soil. A cup of transplanting solution will help plants get off to a good start. Make your own transplanting solution by mixing 1 tablespoons of a water-soluble 20-20-20 fertilizer in a gallon of water.

SOWING SEED DIRECTLY INTO THE GARDEN

Many flowers and vegetables may be sown directly into the garden. Direct sowing avoids transplant shock. It takes less work but involves more risk from weather, pests, diseases and erosion.

Before sowing seeds directly into the garden, know what conditions are required for germination and growth. A warm-season crop, such as beans,

may rot before germinating if planted in cold, damp soil. Knowing the average frost date for your area helps to avoid losing frost-sensitive plants. Some cool- season crops, such as peas and lettuce, should be planted early in the season while temperatures are low.

Sow seeds in a row or broadcast them into a well-raked seedbed. The seedbed should be free of stones or other large debris. Choose a calm day. To broadcast seeds, merely scatter them over a large area in the seedbed. Cover the seeds with a fine layer of soil. To sow very small seeds, mix them with sand before scattering. Then water with a gentle spray. Avoid washing seed away when watering.

SPORES

Ferns can be propagated from SPORES which develop in clusters on the underside of FRONDS. Collect fronds that have produced spores and store them in an envelope until dry. After drying, separate the dust-like spores from the cases by screening. Store spores in an airtight container in a cool, dry place until ready to plant.

Germinating spores requires more time and care than germinating seeds. Growing ferns from spores involves the two different generations of ferns. Spores first produce an asexual plant called a GAMETOPHYTE (gam- EAT-oh-fight). This plant is very small and has none of the usual plant parts. It resembles a moss-like growth and is about 1/8 inch thick.

The gametophyte reproduces sexually and forms SPOROPHYTES (SPORE-oh-fights) which have visible roots, stems and leaves.

During the first phase of growing ferns, sterile conditions are critical. Moss, fungi and algae compete aggressively with young fern gametophytes. Sow spores on top of a pasteurized (sterile), moist, soilless mix or sphagnum peat in a disinfested container. Water must also be sterile. It takes from 3 to 6 months to grow ferns from spores.