BIOCHEMICAL ASPECTS OF SEED VIGOR

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It is beyond the purpose of this presentation to review all the definitions for the term "vigor" as applied to seeds. So far, no one definition has been accepted, and perhaps it is not yet time to settle on one definition until we know more about the subject.

Despite the disagreement on a definition, there is strong agreement on the importance of vigor as a major quality factor of seed. In 1967, the Association of Official Seed Analysts (AOSA) published a survey entitled, *"Seed Technology Research in North America"* and listed research needed in seed technology, but did not specify priorities (12). A questionnaire was then sent out to establish priorities (72). Among the responses vigor was third on the list of the 10 most important areas where research is needed.

Figure 1 illustrates seed vigor in a broad sense. In general, *vigor on the level of an individual seed means one seed producing one normal seedling which will produce a plant of marketable product.* In that respect, vigor is a function of the rate of seedling emergence, of plant growth, and of development in relation to the maximal rate for that variety. Vigor on the level of a population of seeds, may be expressed as *rapid, uniform,* and *high germination or field emergence.* A vigorous seed lot continues to display these 3 properties as planting conditions deviate from ideal to stressful or adverse and thus allows the seed population to maintain *rapid, uniform and high emergence under the broadest range of environmental conditions.* The stresses may include, but are not limited to, extremes of temperature and moisture of soil, high infestation with pathogens, high levels of toxic substances in soil, and unfavorable soil texture.

The degree of tolerance or adaptability of germinating seed to environmental extremes is genetically predetermined, but the variability among individuals within any seed population indicates that many individuals fall short of the maximum genetic potential. In this regard, individuals in any seed lot may fit into 1 of 3 broad categories with respect to their vigor potential: 1) seed that never attained high vigor; 2) seed that attained and maintained high vigor; 3) seed that attained vigor; and then lost it partly or totally. Examples of categories 1, 2 and 3, are as follows: 1) a seed that was harvested at an immature stage and never reached optimum physiological maturity; 2) a seed that developed from a healthy plant under optimum conditions of seed development and maturation, was properly havested at prime physiological maturity, and carefully conditioned and stored under optimum conditions; 3) a seed that reached maximum potential, as in category 2, but lost vigor through damage from delayed harvest, method of harvesting, conditioning, or storage under unfavorable conditions. Seed in category 3 is called deteriorated–a seed that has lost all or part of its vigor. The 3 categories form a continuum: the more vigorous the seed lot, the fewer seed it contains of categories 1 and 3. A seed lot with no seed of categories 1 and 3 would be uniformly high in germinability, field emergence, and seedling growth.

Until now I have discussed vigor as a measurable physiological attribute of seed that is expressed as rapid, uniform and high germination or emergence even under unfavorable conditions. Caution must be exercised in determining the vigor of seed lots that have dormancy at maturity or can be forced into it by subjecting them to stresses, such a high temperature.

At this point, I would like to consider the biochemistry of vigor and review the subject, with emphasis on recently published information. I propose that, on a biochemical level, 3 conditions must be met to attain and maintain vigor: First, a highly organized organelle/membrane system must exist in the seed during seed development; second, disorganization of the organelle/membrane system during seed maturation and dehydration must proceed in an orderly manner such that its reorganization upon hydration becomes possible in the shortest possible time; third, upon rehydration, all membranes must become fully reorganized before the cells become fully hydrated. In reviewing the literature I shall present evidence in support of this hypothesis. We owe most of the recent advances in our understanding of the biochemistry of seed vigor to important advances in membrane physiology and biochemistry. Special recognition must be given to the many horticulturists for their contribution in understanding the physiology and biochemistry of chilling injury in fruits and vegetables in cold storage and the role of membranes in low-temperature tolerance. The information that they generated in studies of fruits and leafy vegetables was extended to other plant tissues, including seeds. Without their efforts, the recent advances in membrane physiology and biochemistry, particularly in the area of low-temperature stress, would have been impossible.

Fig. 1 Vigor differences among lots of soybean seeds of a given cultivar, expressed as differences in emergence, total growth and uniformity among individuals of the plant population.

Vigor and membrane/organelle integrity

The question of membrane integrity is essentially a question of the stability of the 2 major membrane components, the lipids and the proteins, under any specific set of conditions. Both of these components undergo significant changes of 2 types–structural and compositional.

Structural changes. The greatest structural changes in membranes/organelles take place at 2 stages: the first at seed maturation (dehydration) during which seed moisture content drops from 80% or so to 10-12% within a short time; the other at the initiation of germination (imbibition) during which rehydration takes place within a few hours. Both stages have been investigated with the objectives of understanding what happens to the cellular membranes when the seed undergoes these major changes in water potential. However, structural changes of low magnitude also occur when the seed becomes overmature on the plant, when the weather is unfavorable for harvest, and rainy periods alternate frequently with short dry spells. The overmature seed often remains exposed for weeks to these fluctuating weather conditions. While these changes in the field are primarily in response to 1 variable–the change in water potential–the issue is further complicated by other environmental variables, such as temperature extremes that often prevail before harvest and during seed germination.

Combined ultrastructural and physiologial studies provided valuable information on major structural changes that take place in seed membranes during seed development, maturation, quiescense and subsequent germination (6, 12, 28, 36). All these studies agreed that the organization of the membrane/organelle systems was maximal during seed development. As the seed matures, the organelles begin to lose their structural organization in varying degrees and become less active metabolically. By the time the moisture content reaches 12 to 14% (characteristic of a dry seed at maturity) the cells and organelles show signs of shrinkage and alterations of membrane structure and permeability (27). The rough endoplasmic reticulum is reduced to a few crescent layers in the region of organelles and plasmalemma. The cytoplasm contains a few ribosomes, and few or no free polysomes can be detected. The mitochondria have a simplified structure with an electron-dense matrix and few cristae. The golgi apparatus and the rough endoplasmic reticulum disappear and the spherosomes (oil

bodies) align themselves along the plasmalemma and around other cellular organelles. This situation is maintained until after several hours of imbibition.

In spite of the agreement among most investigators on the interpretation of electron micrographs, Luzzati and Husson (40) raised serious doubts about the validity of information drawn from ultrastructural studies. They based their doubts on their observation that changes in the thickness of the water layer around the membrane and/or changes in lipid concentration can alter the structure and in turn the functions of the membrane. Such changes indeed take place when the tissue is dehydrated during fixation. As a result, structural changes in the membrane occur and these changes must be viewed primarily as artifacts of fixation techniques.

Simon (61) described membrane changes that might occur in relation to seed hydration and dehydration. In a hydrated cell, membrane phospholipids (such as those of the plasmalemma) are lined up side by side to form a bilayer. The hydrophobic tails form a nonpolar region in the center, and the polar head groups face the aqueous region on either side. Thus, it is primarily the nonpolar central region that serves as a barrier to diffusion across the membrane. Embedded in the membrane are globular proteins, some of which are enzymes involved in active transport. As Simon pointed out, the structural organization of the membrane depends on the tendency of the nonpolar groups to exclude water and locate themselves in a nonpolar enclave in the center of the membrane. At the same time the polar head groups of the phospholipids and the charged groups of the proteins face the water at the membrane surface. The presence of aqueous phases on either side of the membrane, as in highly hydrated tissues, "forces" the membrane lipids into this lamellar configuration, assuring its structural and functional integrity.

As for the minimum amount of water needed to maintain the membrane in an orderly form, i.e. maintain a lamellar configuration, work on membranes from brain showed that a minimum of 20 to 30% water was essential (40). When the water content fell below that range, a major reorientation of the phospholipids took place. The head groups arranged themselves along water-filled channels in a predominantly hydrophobic matrix. In this hexagonal configuration imposed by low moisture, the membrane did not maintain a continuous boundary. Rather, it contained water-filled channels and became ineffective as a barrier. Unfortunately, such information as derived from thermal analysis and X-ray defraction techniques, is not available on membranes from seeds. However, Pollock's (52) observation that raising the moisture content of lima bean axes to 20% or higher protects the membranes from low-temperature during early imbibition, is in line with the findings of Luzzati and Husson. The requirement for a minimum of 20 to 30% moisture by the brain tissue to maintain the orderly lamellar configuration and the same requirment by the lima bean axes to escape chilling injury and allow minimum leakage of solutes, is too good an agreement to be viewed as a mere coincidence. Possibly, membranes of seeds, like those of other tissues, display polymorphism and thus moisture is a major determinant of their structural configuration.

Compositional changes. In the old literature, cellular instability at high temperature was attributed to the coagulation of protein, destruction of enzymes, and asphyxiation (18). A "lipoid liberation" theory was also advanced. Accordingly, heat injury, whether reversible or irreversible, was attributed to the "melting" of lipid constituents in the cell or in the cell membranes. This idea is linked with the observation that lipid formed by living organisms at high temperatures is more highly saturated and solidifies at a higher temperature than the lipids formed at lower temperatures. It has been further suggested that cells cannot grow at temperatures below the solidification point of their lipids and that plants grown at low temperature must contain more highly unsaturated fatty acids than those grown at optimal temperatures for growth.

Changes in lipid composition of plant tissues under specific stress conditions have been reported (24, 41, 68). Lyons and Asmundson (41) concluded that, in general, chilling-sensitive species possessed mitochondria with a higher amount of saturated fatty acids in their mitochondrial membranes than chilling-resistant species. De La Roche et al. (24) observed that changes in total lipids and total fatty acids of winter seedlings grown at 24°C and 2° were similar at comparable stages of morphological development. In contrast, the phospholipid content was considerably higher of seedlings grown at 2° than of seedlings grown at 24°. Wilson and Crawford (68) reported that the degree of unsaturation associated with the phospholipid fraction from leaves increased in chill-resistant and chill-sensitive species after seedlings were exposed to low temperatures. In their experiments, cold treatment increased the degree of unsaturation of the phospholipids, which are only a small component of cell membranes in terms of total fatty-acid composition. These results suggest that only a portion of the membrane lipids has to be altered to lower the temperature to which plant tissues can be subjected without damage. Those studies related not only to the genetic control of chilling sensitivity but also to seed and seedling vigor when germination in initiated under low-temperature stress.

Pea seeds that had deteriorated because of subjection to high humidity and high temperature responded with a decrease in the linolenic acid content (30). Infection of the seeds with *Aspergillus ruber* caused no further reduction in the level of this unsaturated fatty acid. Since the level of saturated and monoenoic acids did not change with the aging treatment, Harman and Mattick (30) concluded that β-oxidation did not occur and that the reduction in linolenic acid and vigor were suggestive of free radical formation due to peroxidation of fatty acids. Advancing this hypothesis one step further, Pammenter et al. (50) reasoned that since free radicals are charged molecules, the application of a continuous source of electrons during the accelerated aging process should prevent free radical formation and, in turn, preserve viability. In fact, the authors did preserve viability under accelerated aging conditions by providing corn seeds with a continuous flow of electrons from the cathode of a 300-volt current.

The responses of both pea and corn seeds to accelerated aging suggest that lipids are oxidized. In contrast, soybean seeds responded to the same stress by only a slight increase in total lipids and a slight decrease in phospholipids (54). The levels of unsaturated fatty acids present in the whole seed showed no decline during aging. Similarly the fatty acids present in the polar lipid extract from the whole seed showed little change in unsaturation. The authors concluded that oxidation of seed lipids may be unrelated to the process of seed aging.

Some of the above theories originated from studies on cells of animals and of plant tissues other than seeds, but there is evidence that they apply to seeds to a certain extent. However, caution is necessary in extrapolating from animal and plant membranes in general to seed membranes. This conclusion, as suggested by Simon (61), is based on major known differences between seeds and other plant tissues. Most growing plant tissues (roots, fruits, leaves) whose membranes were investigated are so highly hydrated that their water content rarely falls below 80% of the total fresh weight. Some processes, such as cell growth and cell wall synthesis, are very sensitive to an increase of a few bars in water stress while other processes, such as stomatal opening, photosynthesis, and respiration, are affected when water potential falls to around −10 bars. Most higher plants are killed by a loss of around 40-90% of their normal water content. In contrast to these tissues, seeds not only survive but maintain viability for longest periods when held at moisture contents ranging between 6 and 10%. According to Edwards (27), the water potential of seeds ranges from −70 to −200 bars. The ability to tolerate this extremely broad range of water potential and to undergo, reversibly, great changes in water potential and in membrane structure, and still survive, is a unique porperty of seeds. This property suggests that seed membranes may have unique lipid compositions.

So far, little has been said about the globular membrane proteins and their integrity under conditions that cause the membrane lipids to undergo structural or compositional changes. Early work (21) suggested coagulation of proteins of stored wheat seed as the major factor in quality reduction. This hypothesis remained inadequately supported by experimental evidence until recent advances in protein chemistry allowed its rigorous testing. In a recent review, Brandt (16) discussed the denaturation of several membrane enzyme proteins between 18° and 0°C. These enzymes include lipase, α-glucan phosphorylase, and mitochondrial ATPase. The increases in activation energy of enzymes at 12°C or lower in chill-sensitive plants, but not in chill-resistant plants, has been interpreted as reduced protein stability due to cold denaturation (67).

Sato and Asahi (58) observed that several enzyme proteins that are associated with mitochondrial membranes of pea seeds detach themselves from the membrane as moisture content of the seed declines, and return to their position on the membrane when seed moisture is increased under proper conditions. This important phenomenon will be discussed later in detail.

The above discussed studies strongly suggest that both lipids and proteins of the membrane become unstable under stresses, such as low tissue moisture or temperature extremes.

Seeds lose water at maturation and reach the critical level of moisture content (20 to 30%), or even lower, before harvest and stay so until imbibition is initiated. They are stored for months or even years at moisture levels of 12% or lower. Membranes in these dry seeds are in a disorganized state and, as such, are inefficient as barriers. The ways in which seed dehydration and rehydration proceed determine how soon the membrane becomes reorganized. The time element

is crucial because the membranes must become organized before the cell is hydrated. If the cell becomes hydrated before its membrane system become reorganized, undersirable mixing or cellular contents, due to lack of compartmentalization, takes place. Some of the cellular components (including membrane consistuents) may even leak from the cell and be lost. Mixing the loss of cellular components have unfavorable consequences one of which might be the reduction or even complete loss of seed vigor.

Changes in specific organelles/membranes in relation to vigor.

In this section specific organelles/membranes will be discussed and structural and compositional changes that may relate to loss of seed vigor will be pointed out.

Plasmalemma and tonoplast. We can safely assume that most inorganic salts, sugars and amino acids in the cell are held in the vacuole. This assumption is true for mature cells in tissues such as endosperm and cotyledon, but not in the unvacuolated cells of the embryonic axis where the cells remain partially differentiated and lack central vacuoles at harvest. It further assumes that the tonoplast and plasmalemma regulate the movements of salts, sugars and amino acids across these membranes such that accumulation of nutrients is maintained against 2 concentration gradients, one across the tonoplast and the other across the plasmalemma. Thus, in a highly functional cell, the integrity of these 2 membranes can be easily measured by determining the efflux of solutes across the membranes into the surrounding aqueous medium.

One possible approach for distinguishing changes in the tonoplast from those in the plasmalemma is based on the use of seed tissues whose cells have not differentiated fully. Cells of these tissues lack the central vacuole, but have a well-developed plasmalemma. Such cells are characteristic of the partially differentiated tissue of embryonic axes (embryo in the case of *Graminea*). Embryonic axes have been widely used in studies of the extent to which the plasmalemma retains salts, sugars and amino acids against a concentration gradient and prevents their leaching from the tissue in response to stress. Two procedures were generally used in such studies: In one, the leaching of endogenous salts, amino acids, and sugars from excised embryos (axes) into an aqueous solution was determined during a unit time at a fixed temperature. In the other, a radioactive isotope (such as ^{32}P), amino acid (^{14}C-leucine), or sugar (^{14}C-glucose) was introduced into the tissue and leaching of the isotope was determined. In the first method, leached endogenous salts are determined by the conductivity method, whereas sugars and amino acids are determined by standard chemical tests (4). In the second method leaching of introduced isotopes is determined by radiological techniques (5, 73). The tissues are then extracted, and retained radioactive material is determined in the extract. The radioactivities in the leachate and extract are used to determine the percent of uptake that had leached by use of the following equation:

$$\% \text{ isotope leached} = \frac{\text{radioactivity in leachate} \times 100}{\text{radioactivity in leachate} + \text{extract}}$$

The isotopic method is so sensitive that it allows determination of leakage from individual axes of soybean (73). In both procedures, correlation was high between leaching of solutes and seed vigor. In general, when viability of the seed lot declined significantly, leaching of solutes increased by 50% or more.

Leaching of solutes can be prevented either by imbibition at high temperature or by raising the moisture level of the seed to 20% or higher before imbibing at a low temperature. Either condition satisfies the requirement of the membrane to change from a disorganized (presumably hexagonal) to an organized (lamellar) configuration. If conditions are not proper during the reorganizational period (which calls for water), membrane reorganization is delayed or faulty (15).

It is relatively easy to measure leaching of substances from seeds by the conductance method. In wrinkled-seeded garden peas (*Pisum sativum* L.) high correlations between leaching and reduction in vigor were reported. These results provided the rationale for standardizing, and recommending this procedure as an official vigor test for these species (23 and references therein).

Recently, in a study on soybeans, Yaklich and co-workers (74) examined the practical use of membrane leakage by establishing correlations between field emergence and several vigor tests on the seeds, conductivity measurements were most highly correlated ($r = -.67$) with field emergence each year of the 3-year study.

The extensive data accumulated so far substantiate a significant negative correlation between leaching and vigor. They also provide evidence for a reduction in membrane integrity and suggest structural and/or functional changes in the properties of the tonoplast and the plasmalemma. Nevertheless, one should keep in mind that seeds are subject to many types of mechanical injury that cause bruises, cracks and breakage of tissue. These types of injury increase leakage of material from seeds (4) and should be distinguished from the increased leaching of solutes that becomes noticeable when properties or functions of membranes undergo a change.

So far, focus has been on the plasmalemma and tonoplast as barriers whose major role is to confine cellular contents to the proper compartments. The plasmalemma also has 2 additional major roles that may be important to seed vigor. The first is its involvement in polysaccharide synthesis as evidenced by the association of glucan synthetase activities with plasma membrane-rich fractions (26). The second is its probable function as a site of action for plant hormones (22, 29, 32, 34, 55). With regard to polysaccharide synthesis, I (1, 2) reported that germinating barley and wheat seeds incorporated ^{14}C-glucose into a variety of polysaccharides and that the synthesis of these compounds during early germination declined concomitantly with vigor. In fact, reduction in synthesis of polysaccharides was significant before any reduction in germination, seedling growth, or seed respiration was detected. Based on this observation, I proposed that reduction of polysaccharide synthesis during early germination may be used as a measure of vigor loss in a seed.

As for the role of the plasmalemma as a site of plant hormones, there is evidence that the site of indoleacetic acid (IAA) for growth and/or for transport (22, 29, 55) and the site of abscisic acid (ABA) (32) are located on the plasmalemma. Both hormones, particularly ABA, play major roles in seed germination. However, the relationship between seed vigor and the possible loss in number of sites and/or the loss of specificity of binding of hormones to these sites has not yet been demonstrated.

Mitochondria. Mitochondria are the most studied cellular organelle with regard to seed vigor. The rationale for highly functional mitochondria in a vigorous seed is based on the fact that most of the biochemical reactions that become active upon initiation of germination utilize chemical energy that is conserved in the form of high-energy nucleotide phosphate bonds. A major producer of the most common form of chemical energy adenosine triphosphate (ATP), is the mitochondrion.

Extensive studies on the relationship of mitochondria to seed vigor have taken 2 general approaches: an indirect approach in which whole seed respiration was determined and the consumption of oxygen was correlated with percent and speed of germination and with seedling growth (42, 70, 71); a direct approach in which mitochondrial preparations were isolated, their ultrastructural and/or biochemical (oxidative phosphorylative) properties were determined during early stages of germination, and the results were correlated with seed germination and seedling growth (7). Modifications of the latter approach included the determination of changes in activities of certain mitochondrial enzymes such as dehydrogenases (46 and references therein), changes in the level of ATP (8, 19, 63) and the relationship of these changes to seed vigor.

Several earlier studies showed the importance of respiratory metabolism in the expression of seedling vigor in barley (42), and corn (70, 71). Two recent reviews (46, 69) discussed the relationship of seed respiration and certain respiratory enzymes to seed and seedling vigor. Most studies showed that changes that reduced seed vigor reduced respiration and the activities of respiratory enzymes. These observations emphasized the reduction in activities of respiratory enzymes in response to stress conditions that affected vigor. However, recent investigations by Duke et al. (26) of low-temperature effects on mitochondrial respiration of soybean seeds suggest that they respond to low-temperature stress not only by a reduction in activities of several dehydrogenases, but also by changes in the enzyme complement of mitochondria. The most important changes imposed by low temperature (10° vs 23°C for the control) were in glutamic and malate dehydrogenases, as indicated by differences in Arrhenius plots of activities of these enzymes taken from seeds that had been subjected to the 2 temperatures.

Leopold and Musgrave (37) investigated the nature of damage to the respiratory pathway in soybean cotyledons and axes in response to chilling injury during early imbibition and in response to tissue aging (38). They used the cytochrome oxidase inhibitors cyanide (CN^-) and azide (N_3^-), and the alternate pathway inhibitor salicylhydroxamic acid (SHAM). Their findings suggest that both axes and cotyledons respond to chilling stress by a preferential loss of respiration through the cytochrome oxidase pathway. In the chilled tissue, damage was highest when chilling occurred during the first few minutes of imbibition. A major difference between chilled axes and cotyledons was that the latter showed sensitivity to SHAM in the

absence of N_3^-, suggesting that the cytochrome oxidase pathway was so damaged that the alternate pathway was engaged in respiration. These changes have unfavorable consequences on the efficiency of energy production by the germinating seed. A reduction of 50% or so in the activity of the cytochrome oxidase system (the efficient system in ATP production) means a significant reduction in energy production. This finding is in line with the low ATP level in deteriorated seeds (9, 19, 63). Furthermore, the engagement of the alternate pathway in respiration means the tissues ulitized the less efficient of the 2 systems. Capture of energy per mole of NADPH oxidized is far less *via* the alternate pathway than *via* the cytochrome oxidase pathway.

The above findings are in line with the conclusion drawn by Pollock and Toole (53) that the lima bean seed is most sensitive to chilling injury during the first few minutes of water entry. This is the time that Parrish and Leopold (51) refer to as the time of membrane reorganization.

With regard to the effect of accelerated aging on soybean seed respiration, Leopold and Musgrave (38) reported that unaged seeds utilized the cytochrome and the alternate pathways in the cotyledons but only the cytochrome pathway in the axis. Accelerated aging caused a marked deterioration of respiration, especially through the cytochrome pathway, and there was an associated engagement of the alternate pathway in the seed axis. Based on these results, they suggested that the depressed respiratory activity and the shift in respiration to the alternate pathway might be important in the decline of germinability and vigor.

Results of early studies suggest that the mitochondria in dry seeds and in seeds that had been imbibed for several hours, lack a well developed membrane system (6, 7). Nevertheless, isolated preparations exhibited some membrane integrity and oxidative phosphorylative properties. As imbibition progressed the mitochondria became more efficient in oxidative phosphorylation and their membrane system became more highly developed than that in dry seeds. Respiratory quotient (R.Q) values were high (1.4, or even higher) during the early hours of seed imbibition, declined gradually, and approached unity after several hours. These observations suggest that during early germination the tricarboxylic acid cycle does not function efficiently, and that the glycolytic and the pentose phosphate pathways are major sources of energy. If this interpretation is valid, vigor may then be viewed as a function of time required by the mitochondria become more efficient in oxidative phosphorylation and their membrane system become more highly developed than that in dry seeds. Respiratory quotient (R.Q) values were high (1.4, or even higher) during the early hours of seed imbibition, declined gradually, and approached unity after several hours. These observations suggest that during early germination the tricarboxylic acid cycle does not function efficiently, and that the glycolytic and the pentose phosphate pathways are major sources of energy. If this interpretation is valid, vigor may then be viewed as a function of time required by the mitochondria to become structurally and functionally reorganized so that they, rather than the glycolytic and pentose phosphate pathways, become the main source of chemical energy. Mitochondrial recovery also can be measured by monitoring the time required by the imbibed seed to reach a stable R.Q. value, a value that is determined by the predominant substrate(s) used at that time. A short time requirement to reach a stable R.Q. implies a rapid recovery of mitochondira and, in turn, a vigorous seed. Should this conclusion be true, then the seed lot (within a species) that has the lowest R.Q value at the onset of imbibition would be the most vigorous lot. However, the validity of these proposals must be verified experimentally.

Recent studies by Sato and Asahi (58) on mitochondria isolated from germinating pea seeds showed that, with regard to biochemical properties, there are at least 3 types of mitochondria in dry seeds. These authors demonstrated that mitochondria from dry seeds lack part of their enzyme protein complement. Apparently, when the tissue moisture becomes low, some membrane proteins, such as malate dehydrogenase, become unstable, detach themselves from their position on the mitochondrial membrane, and become dispersed in the cytoplasm. A few enzymes were found in the cytoplasm as long-chain polypeptides with biological activity. During imbibition under proper conditions (such as optimum temperature), these proteins return to their former positions on the membrane. This, in part, explains why mitochondrial membranes appear more highly organized in the seed after 6 hours of imbibition, than in the dry seed, even under conditions that do not allow *de novo* synthesis of proteins.

The transformation of mitochondria from inefficient to efficient organelles is part of the membrane reorganization that takes place upon hydration of seeds. Bramlage et al. (15) pointed out that without proper conditions during imbibition, membrane organization may be faulty and the membrane loses efficiency. By faulty organization, the authors meant that the proteins and lipids would not return to their specific positions on the membrane. As a result, the functions of the membrane would become impaired.

Most studies on seed respiration and respiratory enzyme activity focused on the earliest degradative processes associated with decline of seed vigor, but one series of studies put forth the positive concept of mitochondrial heterosis (44, 45, 57). This concept is based on the functional superiority of mitochondria from heterotic hybrids (measured as respiratory rate and efficiency of oxidative phosphorylation) as compared with mitochondrial respiration and energy conservation of the parents (43). McDaniel (43) presented an example of hybrid superiority over the better parent, and its correlation with yield in a commercial barley hybrid. In his experiments, seedlings were compared between the hybrid, produced from the cross of barley cultivars '63-j-18-17', and 'Arivat', and the parents. The hybrid germinated and grew faster than either parent and also exhibited a more rapid rate of tissue respiration. When the mitochondria from these barley lines were isolated under identical conditions, the ADP:0 ratios ranged from 2.0 to 2.2 for the parents and from 2.5 to 2.8 for the hybrids. As the ADP:0 ratio is a measure of the efficiency of the mitochondria in conserving energy, the superior efficiency of the hybrid would give it a distinct advantage in rate of seedling growth and vigor. The increased respiration and oxidative phosphorylative efficiency of the hybrid correlated highly with its increased yield over the parents.

The finding of mitochondrial superiority in nature, which was elicited by hybridization, was also recognized in mitochondrial preparations that were isolated from the 2 parents and combined prior to testing their oxidative and phosphorylative capacities. Mitochondrial preparations from the 2 parents, when mixed in a 1:1 ratio, displayed higher respiratory activity and respiratory control than preparations from either parent (43). In fact, they resembled the mitochondrial preparations from the hybrid in these properties. The demonstration in isolated mitochondrial preparations of complementarity that leads to superiority in biochemical functioning, is indeed encouraging evidence of the role of mitochondria in heterosis and vigor. Judging by the available data, the concept might have the potential for use as an *in vitro* test of mitochondrial heterosis and thus of parental combining ability.

Two additional findings support the important relationship between mitochondrial integrity and seed vigor: One was the very rapid and dramatic increase in ATP content of seeds during very early germination (9, 10, 20, 47, 48, 64). The other was the low levels of ATP (19, 63) in deteroriated seeds and seeds of low vigor. The reduction in ATP content of the embryonic axes of soybeans affected the synthesis of proteins and nucleic acids although the various components of the protein-synthesizing system remained functional (8). Apparently, the rate-limiting factors were ATP precursors, mainly adenine, adenosine and adenosine monophosphate. Evidence in support of these observations came from the significant increase (75 to 100%) in ATP level upon the addition of 1 mM solutions of adenine and adenosine into the imbibing medium (9). The synthesis of ATP from these substrates occurred via the nucleotide salvage pathway that apparently served as a major source of adenine and other nucleotides during early germination. The newly formed ATP appeared in a metabolically active pool because it was incorporated rapidly into higher synthesis of nucleic acids. These findings suggest that the inability to sustain ATP synthesis is probably the major cause of the low rates of biosynthetic activity in deteriorated seed and might cause loss of germinability. They do not explain, however, why exogenous applications of ATP precursors into the imbibing axes almost doubled ATP content and stimulated the incorporation of these metabolites into acid-insoluble materials without having a detectable effect on the speed of germination or growth of the axes as determined by measurements of fresh or dry weight of the tissue.

The relationship of ATP content to soybean seed vigor was recently challenged in large scale experiments in which correlations between ATP content of soybean axes and field emergence, stand, and yield were established (74). The results of these experiments ruled out the utilization of ATP content as a measure of vigor because correlation between ATP contents and field emergence were significant only in 1 out of 2 years.

Vigor and proteins.

Development of new cultivars of cereals and legumes of high protein content has had high priority for the plant breeder and is in line with the great need for increasing plant protein sources to meet the need of the world population. Apart from the importance of proteins as a food and feed source, their relationship to vigor has been

explored from 3 aspects: protein content *per se,* stability of the protein synthesizing system, and stability of the active proteins produced.

Vigor and protein content. In 1969, Schweizer and Ries (59) introduced the concept that high protein content in cereal seeds is important as a factor that relates to vigor. They reported that 'Rodney' oat seed, whose protein content that had been enhanced by chemical application of simazine or terbacil to the plant during seed development and maturation, increased grain yield by 21 to 42%. Likewise, wheat seeds that contained extra protein, as a result of field applications of chemicals or of N, developed into larger seedlings. In their experiments, the content of protein in the seed correlated with subsequent growth and yield. The authors suggested that the amount of endogenous protein or of a proteinaceous moiety, that can be controlled may be an important factor in subsequent yield of major agronomic crops. A year later, Ries and coworkers (56) tested the effects of supplemental N and sub-herbicidal rates of simazine and terbacil on growth, yield, and protein content of the second generation of spring and winter wheat. They reported that N increased yield and protein content in 3 out of 4 tests. Sub-herbicidal applications increased protein content in all 4 tests and yield in 2 out of 4 tests.

Lopez and Grabe (39) confirmed the earlier observations (56) that application of high N levels to wheat plants increased the protein content of the seeds from 6.6% to 10.8%. They further observed that, in comparison with average seeds, seeds with high protein content germinated faster and developed into larger seedlings with higher dry matter content only when grown in N-deficient soil. Interestingly, the increase in seed protein content was accompanied by 25% reduction in seed size, namely in reduced starch content of the endosperm. Neither the size of the embryo nor its protein content (42% protein) was affected.

The above observations lead to some interesting conclusions with respect to the proteins of the wheat embryo which are predominantly membrane protein, globulin, and those of the endosperm which, in order of quantity, are prolamine, glutelin, and albumin (35%, 31% and 9%, respectively, of total endosperm proteins) (65). First, the increase in seed proteins due to high N apparently is restricted to those storage protein species abundant in the endosperm and does not affect the proteins of the embryo or of the cellular membranes. The role of these endosperm reserve proteins is important during seedling growth. Their role in seedling growth becomes even greater when seeds are planted in N-deficient soils. This interpretation agrees with the observation of Lopez and Grabe (39) that seeds with high protein content developed into seedlings that were enhanced in size and dry matter content only when grown in N-deficient soil. Second, the correlation between seed density and vigor was observed in seeds of many cultivars of vegetables, flowers and grasses. This relation has long been implemented in selecting rice and lettuce seed of high vigor. The general observation that dense seeds within a size range are more vigorous than light seeds probably relates to the protein/starch ratio of the seed. The density of starch is about 0.8, and that of proteins ranges from 1.30 to 1.45 cm^{-3} (33). Since the increase in protein content of the endosperm is associated with a decrease in endosperm starch (39), any increases in protein content raise the density of the seed. Hence, the trait of high seed density, with high protein and low starch contents, may be the same factor that indicates high seed vigor.

Vigor and the stability of the protein-synthesizing system. Most seeds commonly store more food reserves, including proteins, than they normally need to germinate and to give rise to an autotrophic seedling under ordinary germination conditions in the field. On this basis, I have taken the position that seed vigor is more closely related to the integrity of the protein-synthesizing system, that is, to the ability of the germinating embryo to synthesize new proteins than to the protein content of the seed *per se.* In 1969, I established that one of the earliest biochemical events that takes place when seeds are subjected to unfavorable conditions is the impairment of protein synthesis during the early hours of germination (1). I observed this phenomenon in aged seeds of barley and wheat (2) that had lost vigor, in sunbleached lima bean (3), and in deteriorated soybean (5). On the basis of my early observations, which were later confirmed by others (17, 49, 60), I suggested the use of the impairment of protein synthesis in a seed lot as a measure of the extent of deterioration that the seed lot had undergone (1, 3, 5).

The components of the protein-synthesizing system were studied ultrastructurally and biochemically with the objective of determining the limiting step, event, or lesion that could be the primary cause of reduced protein synthesis and hence a cause of reduced vigor. Review of ultrastructural studies on barley, pea, and lima bean at seed maturation and early germination (6) revealed that in the mature seed the endoplasmic reticulum became frgamented and dispersed in the cytoplasm in the form of short vesicles or crescents that remained associated with electron-lucent bodies. The polysomes were absent and ribosomes in the cytoplasm faded and became less electron dense. This disorganization was more common in the endosperm and cotyledons than in the embryo. The pattern observed in dry seeds also prevailed during the first few hours of germination. In the rye embryo, 6 hours after germination was started, the endoplasmic reticulum, which was predominantly in the form of circlets, proliferated and became closely associated with nuclear membranes (28). From these studies the authors concluded that protein synthesis during the first 6 hours of germination must have occurred either on polysomes within the dense packing of ribosomes or on the circlets of endoplasmic reticulum associated with electron-lucent bodies. On the other hand, protein synthesis during later germination took place on the new ribosomes present on the reticulum that is associated with the nuclear membrane.

Osborne et al. (49), investigated the integrity of some components of the protein-synthesizing system from viable and nonviable embryos of rye grains. Their work focused on biochemical lesions in the transcriptional and translational processes. Their studies of protein synthesis using poly U-dependent, cell-free systems showed that enzymic binding of aminoacyl-tRNA to ribosomes (reflecting transferase I activity) and viability were reduced concomitantly. Nuclease activity increased with aging, while DNA and ribosomal RNA 25S and 18S progressively decreased. Two years later, a close parallel to the impairment of transferase I activity was reported by Bray and Chow (17) and in aged wheat embryo by Dell'Aquila et al. (25) who noted independently the inactivation of the GTP-dependent transferase I that binds aminoacyl-tRNA to the ribosomes. Osborne et al. (49) concluded that failure of protein synthesis in embryos of nonviable grain might have been the cummulative result of a number of different lesions that developed with age and that no 1 lesion was wholly responsible. They further concluded that the loss of vigor that preceded loss of viability could be attributed to impaired DNA templates and to a restricted transcription of RNA that became apparent when the cells again imbibed water on germination.

Vigor and Stability of Proteins. The concept of protein stability in relation to seed vigor is an old one (14, 31). Crocker and Groves (21) suggested that degradation of wheat seeds in dry storage might have resulted from gradual coagulation of proteins. Twenty seven years later, Jones et al. (35) listed 3 major changes in corn proteins during 2 years of storage: decreased solubility, decreased true protein content, and decreased digestibility. The hypothesis of protein instability remained inadequately supported experimentally until the recent advances in protein chemistry. Recent studies suggest that a major factor in low-temperature tolerance of plant tissues is the stability of proteins against denaturation at low temperatures. Wilson (67) pointed out that, in chill-sensitive plants, increases in the activation energy of enzyme reactions at 12°C and below may be the result of protein instability due to "cold denaturation" and inactivation of the enzymes. Brandts (16) listed several examples of low temperature denaturation of proteins, including lipase, α-glycan phosphorylase, mitochondrial ATPase, pyruvate carboxylase, and glutamate decarboxylase. All these proteins undergo cold denaturation between room temperature (18-22°) and 0°C. The author concluded that the generality of this behavior suggests that denaturation must be a fundamental manifestation that depends upon structural transitions common to all denaturation reactions and involves the exposure of non-polar side chains of the protein molecule to the aqueous solvent.

The concept of protein instability fits well with the concept of impairment of the protein-synthesizing system when vigor is reduced or lost. Denaturation of 1 or more enzymes of the protein-synthesizing pathway may lead to an impairment of this vital system.

Plastids. The relationship of plastid membrane integrity to seed vigor has not been adequately studied. Ultrastructural studies on peas (13) lima beans (36) and barley (6) all suggest that this organelle in mature seeds, in general, and in storage tissues, in particular, becomes loaded with starch grains and the membranes disintegrate into fragments.

Possibly, the prediction of photosynthetic potential of the plant through some chemical properties of the proplastids within the embryo of the dry seed should be explored. To my knowledge, this area has not been studied. The feasibility of investigating this area is based on the fact that there is evidence in green-seeded cultivars of lima bean and peas that sunlight, which bleaches the seed, reduces seed vigor (66). As a result of these studies, Wester (66) proposed that chlorophyll content (reflected in greenness of the lima bean cotyledon) might be used as a vigor marker. Abdul-Baki's (3) work on sun-bleached and non-bleached lima bean seeds suggests that chlorophyll reduction may be a sign of damage to membranes in general because membranes

other than chloroplast were damaged in sun-bleached seeds. The observed reduction in respiration and protein synthesis, and the increased leakage of solutes form embryos of sun-bleaches seeds are evidence of reduction in the integrity of mitochondria, endoplasmic reticulum and plasmalemma, respectively (3 and references therein).

Spherosomes. Most of the neutral storage lipids in seeds are confined to the organelle-like structure called spherosome or lipid body. Spherosomes range from 0.2 μm to 1.0 μm in length or diameter, and contain a single membrane that is almost half the thickness of the mitochondrial membrane (11). They are distributed through the cytoplasm of the cell in a hydrated seed. When the seed loses moisture, spherosomes align themselves along the inner surface of the plasmalemma and around other organelles. The membrane-like structure of the spherosome protects the lipids form oxidation and enzymatic degradation.

Wheat seeds, stored under conditions that allowed the growth of the storage fungi group *Aspergillus glaucus,* were examined ultrastructurally (11). The fungus attacked the spherosomes and caused coalescence of these bodies into large masses of amorphous material. These studies were limited, but revealed changes in structural and storage lipids that are associated with loss of vigor.

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GENETIC ASPECTS OF SEED QUALITY[1]

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Quality seed is undamaged, has a high level of germination, and will produce uniform, vigorous seedlings without defects under various environmental conditions.

There has been much work on the physiology of seeds but relatively little on the genetics of various characteristics influencing seed quality. Physiologists have studied the attributes of seed that affect quality and quite often have reported differences among cultivars, but genetic studies are uncommon. However, the physiological studies were necessary before genetic studies could be made; for example, to identify differences among cultivars, to develop techniques which could be used to identify genetically different lines, and to identify characteristics affecting seed performance.

In this paper I will report on various characteristics affecting seed quality and discuss the inheritance of each character as far as possible.

Hard seed.

Seed vigor can be influenced by conditions at harvest, and there are genetic differences in ability of some lines to withstand inclement conditions prior to harvest and after the seed has matured. Hard seed in soybeans (42) has been suggested as a defense mechanism against conditions prevalent during delayed harvest for southern soybeans. Although hard seed is undesirable, its germination after a period of aging was much better than that of the permeable-seeded line.

Some cultivars of beans (*Phaseolus vulgaris* L.) are hard seeded. This character used to be a major problem in beans because it resulted in uneven germination, but it has been bred out of most commercial types, especially of snap beans. Ledebeff (32) indicated that, in crosses between soft-shelled lines and lines with various degrees of hard seededness, the F_1 was intermediate or approached the soft-seeded parent. In the F_2, seed permeability showed all possible degrees of variation between the extremes of the 2 parents. In 1 cross transgressive segregation occurred for both extremes. The data clearly indicated that the degree of hard-seededness was controlled by several genes.

Kyle (30) examined the nature of hard seed in 2 bean cultivars—'Great Northern' and 'Red Mexican'—and discovered that in 'Great Northern' the micropyle was the site of water imbibition while in 'Red Mexican' it was the raphae and hilum. The impermeability of the hilum and raphae was due to a simple recessive gene and was closely associated with the *p* gene for white seed. Gloyer (21), however, showed in a cross of red and white kidney beans that it was quite easy to select for soft seed in white segregants. Radiofrequency dielectric heating has been used for rendering hard seed of alfalfa permeable to water (39).

An advantage of the hard or semi-hard seeded character is that it protects the seed from aging, or, at least, slows the aging process. Selecting for semi-hard seed also may be a means to obtain seed with seedcoats which do not leak nutrients readily during imbibition.

Vivipary. Seeds sprout in the fruits of some tomato cultivars because there is an insufficiency of the sprouting hihibitor abscisic acid (54); UF136 is an example of a severe case. The same problem occurs in beans (43).

Visible defects.

One of the most obvious visible defects in seed is the fish face, or seed coat rupture characteristic, evident in beans. Farooqui and McCollum (19) identified line differences which, under extreme conditions, ranged from 1.5–47.6% seed coat rupture. They observed that progeny of ruptured seed produced no more ruptured seed than progeny of unruptured seed within a given line. Dickson (9) observed a similar range of susceptibility to seed coat rupture, but concluded that there was a single incompletely dominant gene for susceptibility with 25 to 50% penetrance, the latter varying from season to season.

Transverse cotyledon cracking (TVC) occurs widely in large-seeded legumes. McCollum (35) reported that TVC in beans ranged from 0 to 48.3%. Resistant cultivars such as "Cherokee" became susceptible if imbibed with the seed coat removed. This event indicates that rapid imbibition of very dry seed induced rupture, and that a seed coat which only permitted slow imbibition prevented TVC. In other studies (15) the presence of a seed coat did not influence susceptibility to TVC.

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