Physiology and Biochemistry of Seeds

in Relation to Germination

In Two Volumes

1 J. D. Bewley · M. Black

Development, Germination, and Growth

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Dr. J. DEREK BEWLEY, Department of Biology, University of Calgary, Calgary, Alberta, Canada T2N 1N4

Dr. MICHAEL BLACK, Department of Biology, Queen Elizabeth College, University of London, Campden Hill Road, London W8 7AH, Great Britain

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Chapter 1. Introduction

1.1. The Subject Matter of This Book

In this book we will consider the biochemical and physiological phenomena that occur in a germinating seed and the activities that are uniquely related to germination such as food mobilization and early growth of the seedling. It seems clear, furthermore, that in order to appreciate these parts of our subject we need to understand certain aspects of seed development – since what occurs here lays down much of the pattern for later events. Volume 1 of this book is restricted to the above topics. Volume 2 deals with the control of germination by internal and environmental factors, with dormancy, viability and the ecology of seeds and germination.

1.2. The Seed

With the seed the independence of the next generation of plant begins. The seed, containing the new plant in miniature, is equipped with structural and physiological devices to fit it for its role as a dispersal unit and is well-provided with food reserves which sustain the young plant until a self-sufficient, autotrophic organism can be established. The embryonic plant is protected within its coverings, its metabolic activities at an extremely low ebb often not to be re-awakened until some considerable time has passed or a particular environmental stimulus experienced; the new individuals may be dispersed in time as well as in space.

To fulfill its unique role in the plant's life history the seed possesses some special physiological and biochemical properties. Perhaps the most remarkable, and the most obvious, is that most species of seed can remain alive though dehydrated. The water content of the seed may drop to about 10% by weight, many of its cellular organelles becoming disorganized and inactive. In this state of quiescence the seed resists the vicissitudes of the environment but can resume full metabolic activity, growth and development when conditions so permit. An important, fundamental question may, therefore, be asked about the seed: how can the embryo and some of its associated structures, unlike almost all other parts of the plant, withstand desiccation and avoid death? Regrettably, the answer is still not available.

The seed is dispersed from the mother plant endowed with a store of food reserves of protein, carbohydrate and fat in a more concentrated package than occurs anywhere else on the plant. Animals exploit this property when using seeds as an extremely important part of their diet. It is also debatable that civilization began its development when man started to cultivate plants for the food that their "seeds" provided, especially the cereals—wheat and barley in the Near East and Europe, rice in Asia and maize in the Americas. It need hardly be necessary to remind the reader, moreover, that virtually all of man's exploitation of plants in agriculture depends upon seeds—that they can be stored, transported, multiplied and, most important of all, germinated!

Mention of the cereals creates a suitable moment at which to note that many dispersal units which are commonly referred to as seeds are not true seeds at all but single-seeded fruits. In these cases the pericarp remains thin and dry and may even become fused to the underlying testa as in the cereal grains. In other species, e.g. lettuce and sunflower, such fusion does not occur. Physiologically and biochemically these "dispersal units" should be considered as seeds. In this book we have commonly used the term seed, except in the case of cereals whose dispersal units we refer to as grains or kernels.

It is not surprising, therefore, that for the above reasons—biological and agricultural—the physiology and biochemistry of seeds have been intensively studied. This book is an attempt to discuss modern findings on this subject.

1.3. What is Germination?

When a viable (i.e. living) seed is wetted, water is taken up, respiration, protein synthesis and other metabolic activities begin and after a certain period of time the embryo emerges from the seed, generally radicle first: the seed has germinated. Various requirements must obviously be satisfied before these events can occur; in most cases there must be sufficient oxygen to allow some aerobic respiration, and a suitable temperature to permit the various processes to proceed at an overall adequate rate. Many species of seed nevertheless fail to germinate even when these requirements are satisfied. This is because there exists within the seed a block (or blocks) somewhere along the sequence of changes which normally would culminate in protrusion of the radicle through the surrounding structures. These impedances are overcome either by the provision before visible germination of some environmental stimulus such as light or low temperature, or by subtle changes which slowly occur in the seed with the passage of time. This condition—the failure to germinate even under apparently favourable conditions—is called dormancy. But once germination has occurred, growth of the young seedling continues, supported by the mobilization of the food reserves; eventually the plumule is carried upwards and in nature is raised out of the ground into the light where its autotrophic life can begin.

Implicit in the foregoing discussion is the definition of germination which we will use in this book. Germination consists of those processes which begin with water uptake and which successfully terminate with the emergence of the radicle or hypocotyl through the seed coverings. We have taken all events subsequent to this to be part of or associated with seedling growth. Thus, mobilization of food reserves according to our definition is not strictly a component of germination. But clearly, since it is uniquely associated with the germinated seed it is nevertheless best considered in this context.

1.4. How is Germination Measured?

We have seen above that germination can be recognized by emergence from the seed. As far as each individual seed is concerned it is therefore an all-or-nothing event—the seed has, or has not, the expressed ability to germinate. For seed populations though, it becomes possible to mark grades of germination ability (germinability) or capacity, which is simply the maximum percentage of seeds which germinate under favourable conditions. This is not necessarily the same as the germination rate, which is the germination percentage obtained after a certain time under certain stipulated conditions which may or may not be optimum, e.g. in the presence of certain chemicals or at a certain temperature, etc. This is a commonly used measure of germination; reference to the literature will reveal many instances where researchers have expressed their results as the final germination percentage after time, t. The germination rate is, of course, the reciprocal of the "time to germination", another index in common use. This can be expressed for a single seed, for a population, or for a certain fraction of the population, e.g. 50%. Now we have already seen that germination as defined above is really divisible into two parts, *viz* the biochemical preparative processes and emergence itself. Expression of the final germination percentage is informative only of the proportion of seeds reaching the stage of emergence but it reveals nothing about the time taken to reach this stage.

An expression for the mean germination rate of a population was devised by Kotowski [11]. This expresses the so-called coefficient of velocity (C_v) (though not strictly a measure of velocity or speed but of rate) thus:

$$C_v = \frac{\Sigma G_n}{\Sigma (G_n \cdot D_n)} \cdot 100$$

where G_n = number of seeds germinated on day n; D_n = days from initial sowing. Several criticisms have been made of this approach. Heydecker [9] for example, has pointed out that the expression provides no information about the "distribution" of germination, i.e. a certain average rate results when all seeds germinate at the same time or when some germinate early and others very late. Kotowski's coefficient can, however, be transformed into a measure of the distribution of germination ("uniformity of germination") (see [3]). Another device for reducing the contribution of the few, late germinators to the mean rate uses probability graph paper, and plots the cumulative number of germinated seeds against time (see, e.g. [13]).

Realizing the desirability of expressing both the rate of germination *and* the final amount several workers have attempted to derive a single value to combine these two parameters. The "germination value," C, of Czabator [7] can be calculated from $C = pmt^{-1}$, where p is the "peak" germination percent, i.e. the point of inflexion of the curve of germination against time, m = the final germination percent and t = the time for the test. The major deficiency of this method, emphasized by Goodchild and Walker [8] is that C is a value only of the average rate of germination and that identical values can result from several different curves; for example, the time to reach p can be varied without altering mt^{-1}, p or C. An attempt has also been made by Timson [14] to combine rate and final level of germination from $\Sigma\, g_i (t-j)$ where g_i = germination in time interval i (i varies from 0 to t), t = total number of time intervals and $j = i - 1$ [8] Here, too, the result is not completely satisfactory since the same value may be given, for example, by two seed populations one which germinates 90% by the first day and the remaining 10% over the next 9 days, and the other which shows no germination up to the 9th day but on the 10th day 100% germination.

A number of researchers have attempted to overcome these difficulties and include the three factors—total germination, mean rate of germination and variation in the rate—into one description. Goodchild and Walker [8] found it adequate to use polygonial regression methods for curve fitting; whereas Janssen [10] describes a method using the average germination time, the standard deviation and the total accumulated sum of the normal curve. Interested readers should also refer to other papers which discuss the recording of germination data [12].

We must note that measurement of numbers of germinated seeds does not always convey the required information. In certain species radicle growth may commence before this organ visibly bursts through the testa; and in some experiments it may be necessary to pinpoint, to within just a few hours, the time when the radicles first begin to grow. In such cases, the course of germination can be followed by recording changes in fresh weight.

Inspection of the literature will reveal a certain imprecision in expressing the time factor in "germination." Generally, time is measured from first exposing the seed or seed part to water. This is sometimes referred to as the "time of imbibition," a description which is clearly undesirable if we accept imbibition as being only the initial stage of water uptake (Chap. 4). In some cases, "time of germination" is also unsatisfactory; this expression has been used, for example, for isolated cotyledons or even isolated endosperms where obviously no germination in its proper sense can occur. Moreover, according to the usage of "germination" as defined above, only the hours or days up to radicle or hypocotyl emergence from the seed should strictly be classed as germination time—all times after this cover seedling growth. These small difficulties can easily be avoided by reference to, say, the time after sowing or planting, or after the start of imbibition.

1.5. Some Comments on Our Sources

To obtain the material for this book we have consulted a great volume of published literature—research papers, reviews and general works. Each Chapter is extensively referenced, though the reference list generally represents only a fraction of the total source.

In attempting to set down the events of germination in some generally applicable pattern we have encountered certain difficulties. We mention them now not so much as an excuse for the deficiencies of this book but in the hope that researchers and other students in this field might gain from our experience. When trying to construct an overview it is necessary to compare work from different laboratories. The problems we have met in making these comparisons include the following: (1) Various species or different cultivars of the same species are used by different, or sometimes the same workers. (2) Seeds of the same species or cultivar but of different provenances or harvests show variations in behavior. (3) Frequently, the information for a particular species or cultivar is incomplete. For example, much may be known about food mobilization in a species but little about deposition of food reserves; protein breakdown may have been followed in one cultivar, starch breakdown in another and respiration in a third. An overall picture of the sequence of reserve deposition and mobilization therefore has to be constructed from several isolated pieces of information obtained from different species or cultivars. (4) Dissimilar experimental conditions are used by workers investigating the same phenomenon. For example, in studying the breakdown of reserves in isolated cotyledons or endosperm, different researchers have incubated the tissues in various amounts of water on paper, sand, vermiculite or even almost completely submerged. With such a variety of experimental conditions meaningful physiological and biochemical comparisons are rendered difficult. (5) It is often hard to relate the physiological time courses of various sets of experiments to each other; this is because some kind of "marker" has been omitted. If an "event marker" such as time of radicle emergence, increase in fresh weight, appearance of a certain enzyme, start of mobilization of a particular reserve were always included, comparisons among published results could more easily be made.

1.6. Plant Names

Throughout this book we use English and botanical names. Frequently, but not always, both names are employed together. We have assumed that the English names for "popular" plant species are well known even to those readers whose native language is not English and in these cases we often use only the English name; otherwise only the botanical name appears. We hope that the irritation caused by any inconsistencies can be relieved by reference to the glossary of plant names.

Some Articles of General Interest

1. Brown, R.: Germination. In: Plant Physiology. Steward, F.C. (ed.). New York: Academic Press, 1972, Vol. VIC, pp. 3–48
2. Crocker, W., Barton, L.V.: Physiology of Seeds. Waltham, Mass.: Chronica Botanica, 1957
3. Heydecker, W. (ed.): Seed Ecology. Proc. 19th Easter School in Agr. Sci., Univ. Nottingham. London: Butterworths, 1973
4. Kozlowski, T.T. (ed.). Seed Biology. New York: Academic Press, 1972, Vol. I, II, III
5. Mayer, A., Poljakoff-Mayber, A.: The Germination of Seeds, 2nd ed. Oxford: Pergamon Press, 1975
6. Ruhland, W. (ed.): Encyclopedia of Plant Physiology. Berlin: Springer, 1960, Vol. XV/2

References

7. Czabator, F.J.: Forest Sci. **8**, 386–396 (1962)
8. Goodchild, N.A., Walker, M.G.: Ann. Botany (London) **35**, 615–621 (1971)
9. Heydecker, W.: Nature (London) **210**, 753–754 (1966)
10. Janssen, J.G.M.: Ann. Botany (London) **37**, 705–708 (1973)
11. Kotowski, F.: Proc. Am. Soc. Hort. Sci. **23**, 176–184 (1926)
12. Nicholls, M.A., Heydecker, W.: Proc. Intern. Seed Test. Ass. **33**, 531–540 (1968)
13. Roberts, E.H.: In: Viability of Seeds. Roberts, E.H. (ed.). London: Chapman and Hall, 1972, pp. 14–18
14. Timson, J.: Nature (London) **207**, 216–217 (1965)

Chapter 2. The Structure of Seeds and Their Food Reserves

2.1. Seed Structure

In this chapter we will survey the major features of seed structure which should be understood in order to appreciate points raised in subsequent chapters. Detailed accounts of seed structure can be found in works on plant anatomy and morphology as well as in recently published studies [1, 7]. We will, however, give special, detailed attention to the food reserves of seeds, the site of accumulation of which is obviously closely associated with structure.

The seed is derived from the fertilized ovule. In almost all cases the following can be recognized as the fertilized ovule develops: (1) the testa – the product of one or both integuments of the ovule; (2) the perisperm – derived from the nucellus; (3) the endosperm – produced as a result of fusion between one male generative nucleus and the two polar nuclei to form the triploid endosperm nucleus [1]; (4) the embryo – the result of fertilization of the oosphere (ovum) by a male nucleus. The degree to which these various components continue their development or even whether or not they are all retained, leads to some of the fundamental structural differences among various types of seed (Fig. 2.1). In addition, in many species extra-ovular tissue, especially the ovary wall (pericarp), becomes closely associated with the seed during its formation. We should also note the variability in structure even in seeds produced by one plant, i.e. seed polymorphism. Variations in size, presence or absence of endosperm, colour of testa, and amounts of chlorophyll can be found in several species. The factors responsible for producing these differences are incompletely understood.

It is necessary only to remind the reader of the great range in size and shape of seeds, from the spore-like seeds of the Orchids, through the familiar seeds of crop plants (peas, beans, cereals, lettuce, tomato) to the large coconut and huge *Lodoicea* which may weigh up to 15 kg!

2.1.1. The Testa

The testa is generally a hard coat; in some cases a thinner inner testa is present formed from the inner integument. A great deal of attention has been given to the anatomy of the testa and the differences between genera and species are often exploited for taxonomic purposes. Its physiological importance arises from the presence of an outer and inner cuticle, often fatty or waxy, and one or more layers of thickened, protective cells (Fig. 2.2). These features confer upon the testa some degree of impermeability to water and/or gases, including

[1] The "endosperm" of Gymnosperm seeds is haploid megagametophytic tissue.

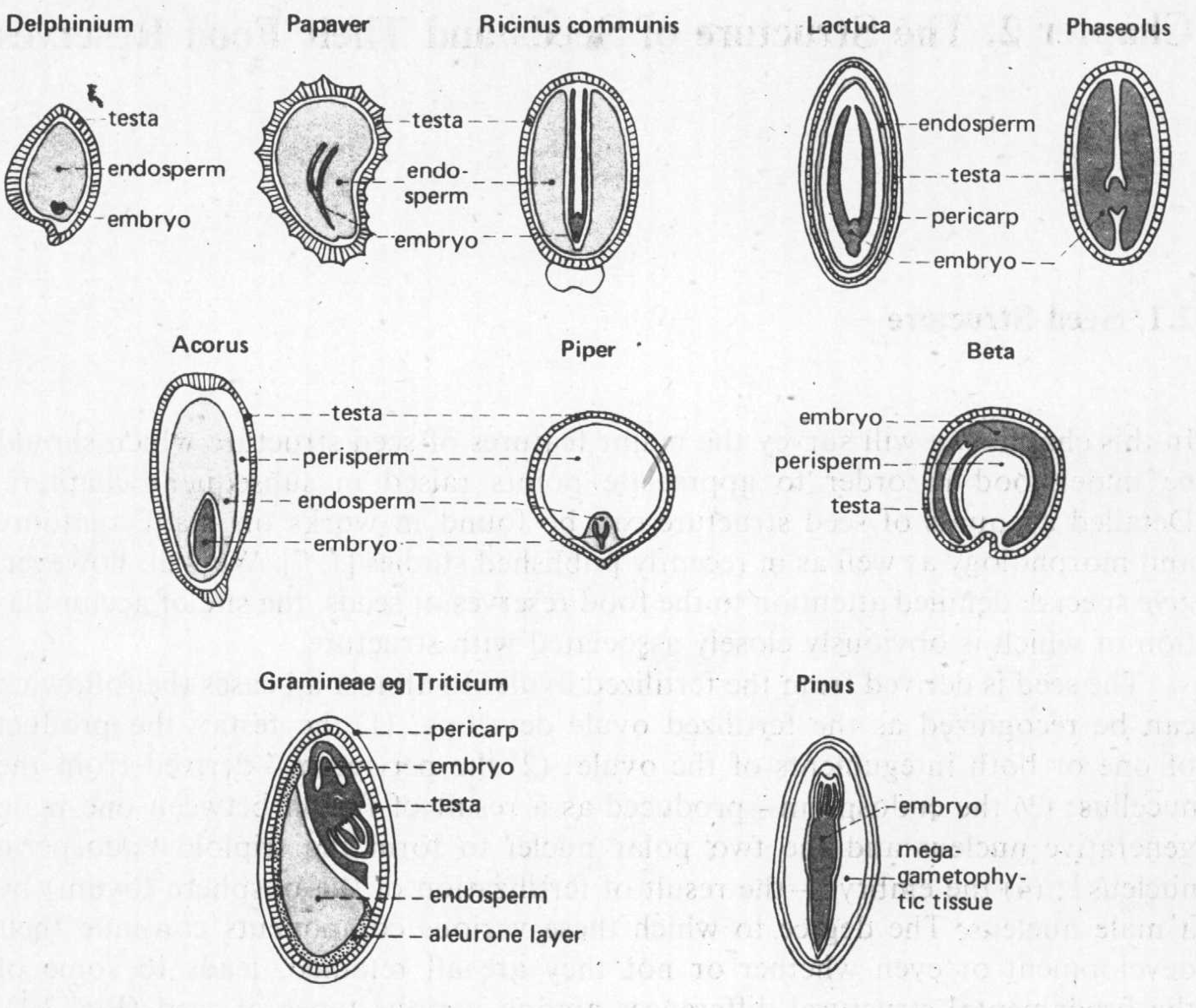

Fig. 2.1. Seed structure. Note the relative proportions of embryo, endosperm and perisperm

oxygen, so that it can consequently exert a regulatory influence over the metabolism and growth of the inner tissues and organs of the seed. In some cases, the testa may be mucilaginous and thereby play an important role in water retention and seed dispersal.

Besides its colouring (sometimes there is a mottled pattern) and texture, an obvious feature of the testa is the hilum. This is the scar, generally of different colour from the rest of the testa and of variable shape and size according to species, marking the point of attachment of the seed to the funiculus. In many seeds a small hole, the micropyle, is at one end of the hilum. The testa of some species, but not many, may have hairs or wings which aid in seed dispersal (e.g. *Epilobium, Salix, Lilium* spp.). Also situated on the testa of many species of seed are outgrowths, such as the warty growth on the hilum named the strophiole; this may be important in controlling movement of water into and out of the seed. Other outgrowths are termed arils. The aril associated with the micropyle, such as the one found in *Ricinus communis*, is termed a caruncle. Arils can take other forms—knobs, bands, ridges or cupules—and are frequently brightly coloured. An aril with which readers may be familiar is that of the nutmeg, *Myristica fragrans*, which yields the spice, mace. Arils in fact frequently contain unusual chemical compounds not found elsewhere

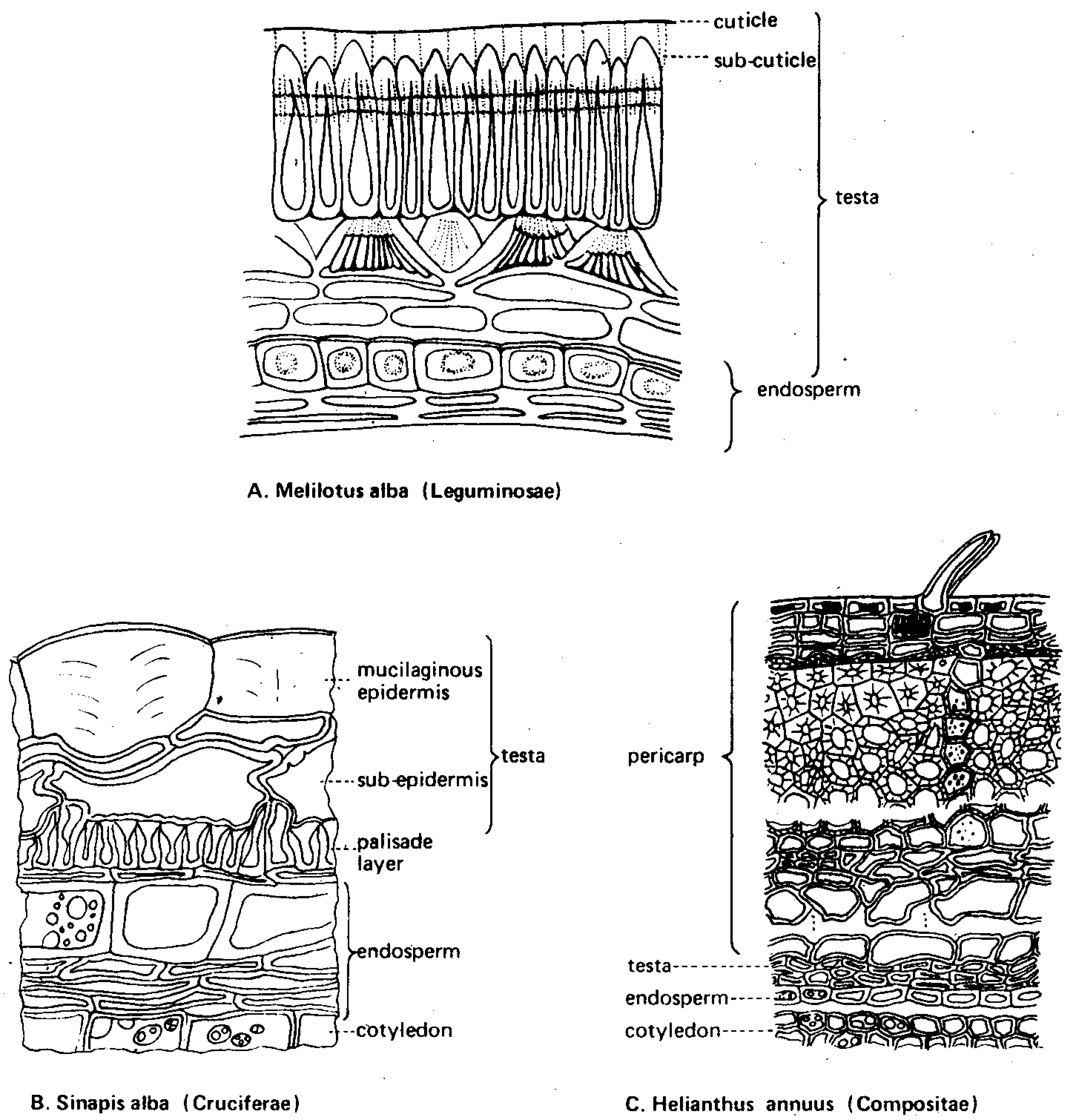

Fig. 2.2A–C. Seed coats. Note the thick testa of the legume (A), the mucilaginous Crucifer (B) and the pericarp of *Helianthus* (C). (A) After Hamly, 1932 [32]; (B) and (C) After Vaughan, 1970 [7]

in the plant. One interesting example is that of *Thaumatococcus* which has an extremely sweet-tasting protein. Arillar contents may be important in attracting animals which aid seed dispersal.

In very many species of "seed" the above features of the "testa" are apparently lacking; this is because the outer coat is not, in fact, the testa but the pericarp (Fig. 2.2C). Many "seeds" whose biochemistry and physiology have been intensively studied are of this type and are therefore truly fruits. The sunflower (*Helianthus annuus*) and lettuce (*Lactuca sativa*) are both cypselas (a type of achene), the cereal grains are caryopses (achenes in which the pericarp