Experimental Embryogenesis in Vascular Plants

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Preface

The study of embryogenesis in plants is of far more than academic interest, since most of the food products used by man are derived from processes related to embryogenesis and seed formation in higher plants. The survival of man and his civilization depends largely upon his ability to circumvent the normal reproductive processes in plants and thereby produce an abundance of food, efficiently, dependably and cheaply. To a considerable extent, practical methods to improve the quality of our agriculture have their origins in fundamental principles discovered in the laboratory, and therefore an understanding of those principles is likely to enrich our attempts in the field. These considerations, as well as the fact of my botanical interest being largely confined to the reproductive processes in vascular plants, prompted me to bring together in a comprehensive form an account of the experimental investigations on embryogenesis to reflect the great expansion in research that has taken place in this area during the last decade.

The growth and development of embryos is an aspect of plant science of considerable contemporary interest and constitutes one of the main focal subjects within the whole area of plant development. Historically, as was the case in many areas of botany, the study of embryos began as a descriptive field of study. Gradually, interest in embryogenesis has evolved in a direction utilizing modern techniques of tissue culture, cytology, physiology and biochemistry and unified by the thread of relationship between structure and function. This functional approach is concerned with an understanding of how embryos grow, how growth mechanisms determine their structure, and how these mechanisms can be modified. In more recent years, the strategy in the study of embryos has been completely changed by discoveries relating to the concept of totipotency and the production of somatic embryos from plant cells. The major emphasis in this book, which simply reflects an exciting era of research in developmental botany, is centered about these dynamic activities of embryos as they develop from their single-celled origin and interact with their milieu. In writing this book, I have in mind advanced students, professional workers and teachers including agronomists, foresters, horticulturists and plant physiologists interested in the study of embryogenesis in plants. I believe that this book can help students and research workers in these fields by providing them with a ready source of information on experimental studies on plant embryos. Some of the concepts described herein have practical overtones which will be of use to those wishing to apply them.

The sources of illustrations used in this book are credited in the captions that accompany each. However, here I wish to express my appreciation to my professional colleagues who kindly provided photographic prints from their works for inclusion in the book and to their publishers, too many to list here, who generously gave me reproduction rights for illustrations and tables used here. Although a succession of secretaries have typed drafts of this book, the final version was typed by Ms. Mary Malone and Ms. Pat Walker, to whom I express my thanks for their careful and conscientious effort. I am also indebted to my wife. Lakshmi, who gave up other interests to translate articles from French for my use. This book has been in the making for a long time. During this period, when I was spending untold hours in my office, I was supported by my family, without whose understanding and fortitude it would not have been possible to complete this work.

June. 1976 V. RAGHAVAN

Abbreviations

The following abbreviations are used in this book:

AMO-1618 = 4-hydroxy-5-isopropyl-2-methylphenyl trimethylammonium chloride, 1-piperidine carboxylate
AMP = 3′,5′-adenosine monophosphate
ATP = adenosine triphosphate
2,4-D = 2,4-dichlorophenoxyacetic acid
DNA = deoxyribonucleic acid
GA = gibberellic acid
GTP = guanosine triphosphate
IAA = indoleacetic acid
IBA = indolebutyric acid
NAA = naphthaleneacetic acid
NAD = nicotinamide adenine dinucleotide
NADPH = nicotinamide adenine dinucleotide phosphate
RNA = ribonucleic acid
mRNA = messenger RNA
rRNA = ribosomal RNA
tRNA = transfer RNA
TIBA = triiodobenzoic acid

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1. Experimental Plant Embryogenesis: Problems and Prospects

The seed germinates heralding the active life of the plant. Given an adequate supply of water and mineral salts in the soil, the normal composition of the atmosphere, a favorable temperature and the energy of sunlight, the seed will form the seedling, the seedling will mature into the adult which will eventually flower and set seeds for the next generation—all on a predictable schedule. If we trace the history of the adult plant backwards through time we see that the master unit in the seed from which it has evolved is the germ or the embryo. The embryo thus represents the beginning of the new sporophytic generation. Even in an immature seed the embryo is sufficiently well developed with morphologically differentiated primordia of the future vegetative organs of the plant, namely, the radicle or the embryonic root, and the epicotyl or the embryonic stem which in many cases may already have the rudiments of the first pair of leaves. The process of germination is the outward and visible evidence of the innate capacity for growth of the different parts of the dormant embryo which depends upon subtle alterations in cell control mechanisms. Most of us are aware of, or have seen, the dramatic sequence of events from the time a seed is planted in the nursery bed to the point when the seedling appears above ground. Not visible to the naked eye, but nonetheless important, are several hormonal substances whose marvelous interplay controls and directs the organized growth of the plant from the embryo. Evidence for this generalization is overwhelming and some of the critical proofs are landmarks in plant physiology literature.

While plant physiologists and developmental botanists have succeeded to a remarkable degree in unraveling the causative factors that regulate growth of the component parts of the plant, surprisingly meagre and primitive is our knowledge of the determining factors in the organized growth of the embryo in undisturbed normal development. In both plants and animals, the fertilized egg or the zygote is the fundamental structural unit which, by a series of subtle and complex influences, becomes progressively expressed to give rise to the fully developed embryo. There is an extreme contrast between the zygote and the embryo that develops from it. The zygote is best appraised by stating that it is an inherently unstable entity: it maintains its seemingly simple organization only for a brief period of time before it subdivides into a large number of smaller cells. These cells group into self-contained pockets of tissues which in turn differentiate organs. Thus, out of a simple-looking cell emerges, by a series of developmental events closely coordinated in time and space, the complex embryo, endowed with an array of distinct parts, each destined for a specific function in the adult. Perhaps in no other organ in the plant do we find such dramatic changes in growth, differentiation and tissue formation crowded into a relatively brief span of time as in the embryo.

Biologists have often sought to answer the question: What causes a single-celled zygote to reproduce meticulously, generation after generation, a complicated pattern of many cells which are biochemically and structurally so unlike itself? It has proved no easy matter to answer this question without indulging in philosophical generalities, in spite of the fact that a considerable part of the contemporary effort in biology is dedicated to questions of this nature. It is reasonable, although a gross oversimplification, to assume that the genetic information built up in the zygote determines the specific pattern of cells that subsequently arise from it. As all the cells formed by the division of the single-celled zygote receive the same genetic blue-print and thus conserve the characters and potentialities of the parent cells, we are further provoked to ask, without being able to give the answer: What factors order or stimulate one type of growth in certain cells, and another type of growth in certain other cells of the same organ?

This book does not claim to be able to answer these questions and perhaps many others of equal significance which have aroused widespread and general concern among biologists for some time now. What I intend to do is to bring together some of the findings from the literature in the experimental embryogenesis of vascular plants (pteridophytes, gymnosperms and angiosperms), both past and present, in the hope of stimulating the kind of research that will answer the questions in future.

What is experimental plant embryogenesis? So far the greatest effort in plant embryology has been directed towards descriptive accounts of sporogenesis and gametogenesis and enumeration of the steps in the transformation of the zygote into a fully fledged embryo, by precise observations of histological preparations of the male and female gametophytes and embryos of different ages. This work, described in some classical books (Coulter and Chamberlain, 1917; Campbell, 1930; Schnarf, 1929, 1931; Bower, 1935; Eames, 1936; Johansen, 1950; Maheshwari, P., 1950) and scores of scientific papers, has been carried forward by many investigators in many laboratories around the world; it has as its major goal an understanding of the architectural principles on which gametes are formed and embryo types are constructed. These studies provided a useful framework for an appreciation of the range of variation in the development and organization of gametophytes and embryos of vascular plants and created a deeply entrenched idea that some of these events in each plant follow a typical pattern according to a blue-print characteristic of each species.

Evaluation of the accumulated data relating to sporogenesis, gametogenesis and the mode of embryo formation in a large number of species led to a diversification in the outlook in plant embryology. The utilization of embryological data in assigning phylogenetic affinities of certain families, genera and species of flowering plants was the immediate outcome of such diversification (Maheshwari, P., 1950, 1964; Johri, 1963b). Numerous and valuable indeed are the papers which describe gametophyte development and embryogenesis in the different species of plants and application of the data to solve phylogenetic problems and taxonomic relationships.

Since 1930, advances made in the fields of plant physiology, biochemistry and genetics, and refinements in the culture of plant organs and tissues under aseptic conditions, have had much influence in the orientation of modern order in plant embryology. This has given rise to the comparatively new discipline of experimental embryology, involving control of pollination and fertilization and manipulations of the anther, pollen grain, ovule, ovary, and embryo, by excision and culture, by chemical, hormonal and surgical treatments, and by exposure to selected day-length and temperature conditions to study the controlling mechanisms that affect their form and structure, in the hope that as we gain knowledge of the laws that control the unfolding of form and structure in the reproductive organs of plants, we may get new clues to control them to our advantage. Thus experimental embryology has changed an era of observations and inferences into an era of experiments and deductions, designed to discover what processes are involved in the evolution of embryonic form, how they are related and how they are controlled.

The boundary between embryology and embryogenesis seems to be somewhat vague. In contrast to embryology which includes all of the events connected with microsporogenesis, megasporogenesis, development of the male and female gametophytes, fertilization and endosperm and embryo formation, embryogenesis (embryogeny) is concerned with the whole constellation of post-fertilization events, and is regarded as the continuum of processes involved in the origin, growth, and orderly transformation of the zygote into a fully fledged embryo. With this limitation in mind, I have followed an orthodox approach in the text, beginning with a consideration of the formation of the zygote and ending with the embryo complete with all of its tissues and organs, emphasizing experimental studies which provide causal explanations of growth, development and morphogenesis during embryogenesis. Although experimental work on plant embryos is in its very embryonic stages, a survey of the work done so far, summarized in some recent reviews (Narayanaswami and Norstog, 1964; Maheshwari, P. and Rangaswamy, 1965; Wardlaw, 1965b; Degivry, 1966; Raghavan, 1966) will unfold the challenging future opportunities which will keep plant embryologists occupied for a long time.

In summing up, experimental study of the formation of the embryo, its nutrition, its responses to external stimuli and modification of its growth by hormonal and environmental factors, is the main theme of this book. The development of the text can be briefly summarized as follows. In the first three chapters, I have closely examined the present state of our knowledge of the structure, growth, and organization of the egg, zygote and embryo in representative species of vascular plants. The background of events leading to the formation of the embryo thereby established serves as a basis for the subsequent two chapters on the nutritional aspects of embryogenesis. The next six chapters are concerned with growth, organization, and differentiation of the embryo during progressive embryogenesis and its changing patterns of nutrition and metabolism. Although the framework of discussion is provided mainly by vascular plants, significant contributions from other taxonomic groups are stressed, especially when they illustrate principles that are more generally applicable. Included herein toward the end is also an account of the major advances made in the past few years in the applied aspects of embryo culture and in the experimental production of adventive embryos. Although the metabolism of the embryo during storage and germination of seeds is outside the scope of this book, some aspects of the physiology of the dormant embryo and the developmental and biochemical aspects of germination are discussed in the final two chapters, insofar as they seemed useful for a comprehension of the initiation of development. Because of the paucity of knowledge, I have not stressed in this book the many intriguing and fascinating problems in differentiation at the molecular level which must certainly be taking place during embryogenesis. Notwithstanding, it is hoped that this book will be of use in bringing together some of the scattered literature on the developmental physiology and morphogenesis of embryos and will give a general idea of the current and future perspectives in the embryogenesis of vascular plants.

Section I

From Egg to Embryo

2. Structure and Organization of the Egg, Zygote and Embryo

Embryogenesis involves extensive changes in form in defined and dramatic ways and a progressive change from the undifferentiated to the differentiated state. Beginning with the first division of the zygote, the plant remains in a state of continuing embryogenesis, producing new cells, tissues and organs throughout its life history. Common to the developmental changes during progressive remodeling of embryo structure are such processes as cell division, cell expansion, cell maturation, cell differentiation and formation of meristems, but the physiological and biochemical changes underlying the histological diversity of organs formed are probably different. Cells produced at each stage of development are arranged according to specific and predetermined patterns to give the embryo certain proportions that remain constant throughout its growth. Changes in the pattern of cell arrangement leading to changes in size and shape of the embryo are determined by the plane of cell division and by the relationship between the frequency of cell division and the rate and direction of cell elongation. The plane of cell division is, in turn, determined by the orientation of the mitotic